

EFFECTS OF POST-EXTRACTION ALGAL RESIDUE ON NUTRIENT
UTILIZATION, CARCASS PERFORMANCE, AND BEEF TENDERNESS AND
FLAVOR IN FINISHING STEERS

A Thesis

by

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ABSTRACT

To investigate the impact of post-extraction algal residue (**PEAR**) on nutrient utilization, carcass performance, and beef tenderness and flavor in finishing steers, eighteen Angus × Hereford steers were used in a three-phase study. Treatments consisted of PEAR hand-mixed into the diet at 1.0 kg OM/d (**PEAR**), 1.0 kg OM/d glucose infused ruminally (**GR**) or abomasally (**GA**). Infused steers were fitted with ruminal cannulae, allowing continuous infusion of glucose via anchored infusion lines. Steers were adapted to housing and basal diet prior to starting; subsequently, treatments were applied approximately 35 d, until harvest. Intake and digestion were determined from d 27 to 31 using fecal grab samples. Steers were harvested on d 34 to 36; 2 d post-harvest, carcass measurements were collected and strip steaks were obtained from each carcass from GA, GR, and PEAR treatments. Three d post-harvest, beef subprimals and subcutaneous fat from the chuck and round of each carcass from GR and PEAR treatments were collected, and 2 d prior to sensory evaluation, subprimals were further processed into ground chuck and round, respectively.

Greater DMI was observed for PEAR (13.0 kg/d) than GR (10.3 kg/d; $P < 0.05$); DMI for steers receiving GA (11.2 kg/d) was intermediate and not different from either PEAR or GR ($P \geq 0.14$). Digestible OM intake was similar among treatments and averaged 8.8 kg/d ($P = 0.51$). Digestion of GE was 72.9, 82.6, and 80.9% for PEAR, GA, and GR, respectively ($P < 0.01$). Steers fed PEAR had greater marbling scores (Mt^{20}) than GA (Sm^{63}) and GR (Sm^{52} ; $P = 0.01$). Accordingly, USDA Quality Grade

was greater for PEAR than GA and GR (Ch^{40} , Ch^{21} , and Ch^{17} , respectively; $P = 0.01$). There was no difference in USDA Yield Grade or HCW between treatments ($P \geq 0.66$). No off-flavors were detected by trained sensory panel analysis in strip steaks from GA, GR, or PEAR ($P > 0.05$). No significant differences for *overall like*, *overall flavor like*, *beef flavor like*, or *juiciness like* were observed in ground round or ground chuck from PEAR or GR fed steers ($P \geq 0.17$).

DEDICATION

This thesis is dedicated to my daughter, Emma Louisa Morrill.

Sweet Emma,

I never want you to be afraid to pursue your goals and dreams—even if they may, at times, seem different or unpopular from those of your peers.

There is no shortcut to excellence. Take time in deciding on the education you want to have and enjoy the journey in pursuing it. Russell M. Nelson once said, “Education is the difference between *wishing* you could help other people and *being able* to help them.” I believe that. Use your education for doing good in this world and always remember why you were sent to this earth—to be an instrument in the hands of the Lord.

All my love,

Mom

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TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
DEDICATION.....	iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	viii
LIST OF TABLES.....	x
CHAPTER I INTRODUCTION AND LITERATURE REVIEW	1
Introduction.....	1
Micro-algae as biofuel source; PEAR as protein source for cattle	3
Feed ingredient selection	5
Cost of PEAR as a substitute for other ingredients.....	7
Ruminant ability to digest things other species cannot.....	7
Inclusion of algae in feed rations of other livestock species.....	8
Finishing beef steers in the United States	9
Protein and PEAR in finishing rations for beef steers	11
Fat content and fatty acid composition of beef.....	12
Fat content and fatty acid composition	13
Marbling or intramuscular lipid	17
Beef tenderness	19
Color and label information effect flavor perception	20
Conclusion	23
CHAPTER II EFFECT OF INCLUSION OF POST-EXTRACTION ALGAL RESIDUE ON NUTRIENT UTILIZATION AND CARCASS PERFORMANCE IN FINISHING STEERS	24
Overview.....	24
Introduction.....	26
Materials and methods	27
Laboratory analyses	31

	Page
Calculations	33
Statistical analyses	33
Results	33
Discussion	36
Conclusion	41
 CHAPTER III EFFECT OF INCLUSION OF POST-EXTRACTION ALGAL RESIDUE IN FINISHING RATIONS OF BEEF STEERS ON STRIP STEAK AND GROUND BEEF FLAVOR.....	 42
Overview	42
Introduction	44
Materials and methods	45
Phase 1: Carcass fabrication, cut selection and storage	47
Phase 1: Sensory evaluation by expert trained panel	48
Phase 2: Carcass fabrication, cut selection, and storage	49
Phase 2: Tenderness evaluation by shear analysis	50
Phase 3: Carcass fabrication, cut selection, storage, and product processing	51
Phase 3: Sensory evaluation by consumer sensory panel	52
Phase 3: Fatty acid analysis by gas chromatography	55
Statistical analyses	56
Phase 1: Expert trained sensory panel analysis	56
Phase 2: Tenderness evaluation by shear analysis	56
Phase 3: Consumer sensory analysis	56
Phase 3: Fatty acid analysis of ground beef	57
Results	57
Phase 1: Flavor of strip steaks	57
Phase 2: Tenderness evaluation by shear analysis	59
Phase 3: Flavor and fatty acid composition of ground beef	59
Discussion	62
Phase 1: Flavor of strip steaks	62
Phase 2: Tenderness evaluation by shear analysis	62
Phase 3: Flavor and fatty acid composition of ground beef	63
Conclusion	66
 CHAPTER IV CONCLUSIONS	 67
REFERENCES	68
APPENDIX	83

LIST OF TABLES

	Page
Table 1. Chemical composition of finishing ration and post-extraction algal residue (PEAR) ¹	28
Table 2. Comparison of fatty acid composition of finishing ration and post-extraction algal residue (PEAR) ¹	29
Table 3. Nutrient utilization of beef steers consuming post-extraction algal residue (PEAR) ¹ or receiving glucose infusion ²	34
Table 4. Mineral and fatty acid intake of beef steers consuming post-extraction algal residue (PEAR) ¹ or receiving glucose infusion ²	35
Table 5. Carcass traits for steers consuming post-extraction algal residue (PEAR) ¹ or receiving glucose infusion ²	36
Table 6. Chemical, macromineral, and micromineral composition of finishing ration and post-extraction algal residue (PEAR ¹)	46
Table 7. Fatty acid composition of finishing ration and post-extraction algal residue (PEAR ¹)	47
Table 8. Consumer panel demographic frequencies reported as percentage of respondents	54
Table 9. Beef flavor attributes of strip steaks from steers that consumed post-extraction algal residue (PEAR ¹) or were infused with glucose. ²	58
Table 10. Consumer sensory ratings for ground beef from steers fed post-extraction algal residue (PEAR ¹) or infused with glucose ²	60
Table 11. Fatty acid composition (% FAME ¹) of ground beef from steers fed post-extraction algal residue (PEAR ²) or infused with glucose	61
Table 12. Sensory attributes, their definitions and their associated references, which are in accordance with the aroma and flavor lexicon used for the trained sensory panel.	84

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Along with a growing and changing population has come an increase in the demand for food. It has been predicted that overall food consumption is expected to increase by 70% and the current supply of meat, milk, and eggs across the globe will need to double to meet demand, by 2050 (FAO, 2006). A major driver of this increase is the expected 167% increase in the global middle class by 2030 according to the World Bank (Bussolo et al., 2008). According to Delgado et al. (2001) consumers in *developed* countries consumed 56% of their dietary protein from animal products while those in *developing* countries only consumed 26%. As countries continue developing, or move more people towards the middle class, demand for animal food products likely increases to levels similar to those of further developed countries. Increased demand for food will need to be met by advances in food production efficiency. While this is a substantial challenge, historical data shows that applying technology and other methods of improving efficiency significantly increased agriculture productivity in the past. For example, between 1961 and 2003 the United States beef industry implemented technologies such as growth implants, ionophores, beta-adrenergic agonists, increased its use of co-products from other industries, and developed feeding practices that more effectively meet animal nutrition requirements. Additional advances came from improved animal genetics, use of EPD based selection, AI, and heterosis from cross-

breeding. Ultimately, the adoption of these practices resulted in a 72% increase in amount of beef produced per unit of land in the United States (CAST, 2012).

Issues facing food producers today and in years to come may include limited availability of land for crop and cattle production, reduced water availability, and greater consumer demand for transparency focusing on environmental and animal stewardship. Additionally, nutrient pricing challenges may arise as industries competing for feed resources, such as corn for biofuel production and swine, poultry, and aquaculture industries continue to grow.

Expanding the current domestic cattle population is not a realistic solution to increase available beef due to the aforementioned circumstances related to available land and water resources, unless other practices such as intensification as suggested by Trubenbach et al. (2014) are adopted. Current limits on national herd size and competition for feed sources, suggest that alternative feed sources need to be evaluated and, subsequently, incorporated into all cattle feeds. In recent years, the beef industry has successfully utilized distillers' grains, a co-product of the ethanol industry, as an alternative feed source for all segments of the beef supply chain.

While the cattle feeding industry is investigating ways to improve or increase production, other industries are doing the same in their respective fields. The biofuel industry, for example, is currently researching micro-algae as a feedstock for biofuel production. A barrier to micro-algae as a biofuel source includes its inability to be cost-competitive with other petroleum-derived fuels (Pienkos and Darzins, 2009). Development of a suitable market for the co-products of algal biofuel would aid in cost

recovery, and result in greater cost-competitiveness to increase the economic sustainability of fuel production while simultaneously capitalizing on the nutrients available in the co-product. The primary co-product from algal biofuel, post extraction algal residue (**PEAR**), contains a high proportion of protein (19-38%; Bryant et al., 2012) and has potential as a possible feed source for beef cattle. However, the market value of PEAR has not been established as no market for PEAR currently exists (Bryant et al., 2012). Using distillers' grains as an example of a biofuel co-product that has been successfully incorporated into cattle feeding, the investigation of PEAR in finishing rations of beef steers will be examined in this thesis.

Micro-algae as biofuel source; PEAR as protein source for cattle

Algal biomass, which has been labeled as a third generation biofuel, while attractive for its energy density (20-75% oil; Christi, 2007) and ability to use land and water resources otherwise unsuitable for food or feed production (Drewery et al., 2014), comes with economic and environmental challenges. Production of biofuel from algae results in a co-product, PEAR, produced in higher amounts than the biofuel itself (Becker, 2007). After lipid extraction, significant quantities of PEAR remain, which is concentrated in protein (19-38% DM basis; Bryant et al., 2012). As the protein content of PEAR closely resembles that of conventional protein supplements for beef cattle, it is suggested that PEAR may be an alternative source of protein in cattle rations, providing an opportunity not only for biofuel producers to generate revenue, but also providing a source of nutrients for beef producers.

In a study conducted by Drewery et al. (2014), provision of PEAR as a protein source to cattle consuming wheat straw, compared to supplemental cottonseed meal, resulted in similar increases in straw intake and digestion. Supplementation with increasing levels of PEAR resulted in quadratic increases in both straw OM and total digestible OM intake (**TDOMI**). Intake of TDOMI peaked when 100 mg of N/kg of BW was provided; however, the optimum level of supplementation may reside between 50 and 100 mg of N/kg of BW, because levels of PEAR greater than 50 mg of N/kg of BW decreased NDF digestion. Providing PEAR as additional protein in small amounts is more efficient than providing PEAR at greater amounts, which agrees with observations of Sawyer et al. (2012), suggesting that providing small amounts of supplemental protein will increase forage utilization.

Post-extraction algal residue does, however, present challenges to beef cattle feeders. While PEAR does contain a high proportion of protein, it can also contain high levels of ash (up to 46% ash; Bryant et al., 2012). Additionally, its physical form poses another challenge for beef producers. In previous research using PEAR as a feed source for beef cattle, it was necessary for moisture to be added to the PEAR to decrease dust and to allow PEAR to stick to other feed ingredients (Wickersham, 2014). Wickersham (2014) also successfully pelleted PEAR to improve its handling characteristics. Furthermore, PEAR samples have contained minerals at levels of concern for producers that could pose ration formulation challenges or toxicity concerns.

According to Bryant et al. (2012), toxicity from macrominerals by PEAR is not expected as PEAR does not exceed maximum tolerable levels nor is PEAR expected to

be included in a ration at high enough concentrations to pose threats for micromineral toxicity because of its high ash, salt, and/or protein content. However, one concern of Drewery (2012) was the mineral and heavy metal concentrations of her PEAR samples, when comparing samples of different algal species, methods of processing, etc., to conventional protein supplements and maximum tolerable concentrations of minerals for beef cattle (NRC, 2000). In Drewery (2012), PEAR samples contained S (0.83-0.85% DM in diatom PEAR, 0.87-0.93% DM in flocculated PEAR) and Al (288-319 ppm in diatom PEAR, 4130-4310 ppm in flocculated PEAR) at levels in excess of maximum tolerable concentrations (if considered as a complete diet), but when PEAR was fed as a protein supplement to cattle consuming low-quality forage the relative amount of PEAR fed was diluted, negating toxicity concerns.

Feed ingredient selection

One way to increase efficiency in beef production is by improving the precision of diet formulation. Diet formulation goals for beef cattle include providing ingredients at optimum levels to meet nutrient requirements for maintenance and growth, while also maintaining the integrity and quality of beef products. Additionally, economics, including cost and geographic availability of ingredients, need to be considered before deciding upon the inclusion of feedstuffs into beef cattle rations. One method of determining whether cost of ingredients is economically feasible is by examining cost of gain (**COG**). Cost of gain is examined to determine if feeding programs are appropriate, given market and environmental conditions. Cost of feed ingredients plays an important role in purchasing decisions of beef producers because feed ingredients directly affect

COG. If COG is high relative to value of gain (**VOG**) a change in diet formulation is necessary.

Changing diet formulation creates both opportunities and challenges for producers, however. Feed ingredient selection and level of inclusion not only impact diet palatability, animal performance, and COG, but also can effect beef quality. Even changes to like feed ingredients, such as processing methods, can cause differences in animal performance. For example corn can be processed in multiple ways for feeding to livestock, including but not limited to steam-flaking and dry-rolling. Steam-flaked corn vs. dry-rolled corn, when included in finishing rations of beef steers, reduced DMI by 9% ($P < .10$) and increased feed efficiency by 14% ($P < .01$) when fed at equal quantities on a DM basis (Barajas and Zinn, 1998). This difference in processing impacts how the ruminant animal is able to digest and utilize the feedstuff. Also, according to the NRC (2000), dried distillers' grains contain approximately 30% CP and 10% fat; however, Saunders and Rosentrater (2009) found that dried distillers' grains that undergo further processing to recover additional oil contain 33-35% CP and 2.3-3.4% fat, potentially impacting the feeding value of distillers' grains due to the change in protein and fat content. So in the case of PEAR, different processing methods could potentially create differences in nutrient utilization if production of PEAR on a large scale is not done in a consistent fashion, and protein, ash, and fat content varies. Additionally, variance in species of algae could also potentially result in varying nutritive value and thus utilization of PEAR.

Due to the nature of oil extraction during biofuel manufacture, it is expected that the remaining PEAR fraction will contain a high proportion of protein (19-38%; Bryant et al., 2012). As the protein and energy portions of beef cattle diets are costly components of rations, it is likely the cattle industry will be open to considering PEAR as an alternative source of protein/energy for cattle. As PEAR is being considered as an alternative source of protein/energy for cattle, the impacts of increased protein/energy levels from PEAR in the ruminant diet need to be considered.

Cost of PEAR as a substitute for other ingredients

From 2005 to 2010, the average prices of high-protein soybean meal, low-protein soybean meal, and cottonseed meal were \$256, \$244, and \$208 per ton, respectively (Feedstuffs, 2005-2010). Post-extraction algal residue could have been considered as an alternative protein source for soybean meal and cottonseed meal to decrease COG according to a hedonic pricing model for PEAR suggesting that PEAR would have been valued between \$100 and \$225 per ton from 2006 to 2010 (Bryant et al., 2012). If PEAR were to have been priced below \$208 per ton, PEAR would have been a more cost effective feed ingredient in terms of COG compared to its soybean and cottonseed counterparts, and therefore would have deserved consideration as an alternative feed ingredient, if diet utilization is similar.

Ruminant ability to digest things other species cannot

Ruminants, unlike swine and poultry, use the rumen to convert low-quality by-products from other industries, crop residues, and forages, into nutrient rich, edible, protein sources fit for human consumption. The rumen is relatively efficient at digesting

cellulose, which is the most abundant plant product on earth (CAST, 1975), but is indigestible by mammalian enzymes. While PEAR is not expected to contain high quantities of cellulose, it remains unlikely for PEAR to directly serve as a source of human food because of its color, smell, and flavors, which are associated with algae (Becker, 2007). The ability of beef cattle to effectively consume and digest PEAR highlights the uniqueness of ruminants and their ability to convert compounds inedible, indigestible, or unpalatable to humans into an edible, palatable, protein source (i.e. meat).

Inclusion of algae in feed rations of other livestock species

Algae has been included in the diets of other livestock species, such as pigs and poultry to change meat composition and as an alternative feed source. For example, in an effort to improve the iodine content of pork, iodine-rich algae was included in the diet of pigs. According to the study, iodine content of pork was increased up to 45, 213, 124, 207, and 127% in fresh muscle, adipose tissue, heart, liver, and kidney, respectively ($P \leq 0.05$; He et al., 2002). Additionally, it has been suggested that algae could be used as a partial replacement of conventional protein sources for poultry (Spolaore et al., 2006), however, algae is likely overlooked as a feed source due to the large amount of algal biomass that would be required to feed large amounts of livestock (Kovač et al., 2013). Large amounts of PEAR that would result from biofuel production; however, would combat the issue mentioned by Kovač et al. (2013).

An algae meal co-product of biofuel production (*Chlorella* sp.) was included in the corn-based diet of finishing barrows in a study by Dib (2012); HCW decreased with

increased level of algae meal inclusion from 39.6, 36.8, 35.1, 32.8, and 29.8 kg for 0, 5, 10, 15, and 20% inclusion level, respectively ($P < 0.01$). Additionally, marbling score numerically increased to 1.87 and 1.72 when included at 5 and 15%, respectively, compared to 1.48 in controls receiving no algae-meal. The results suggest that feeding algae meal (*Chlorella* sp.) up to 20% to finishing pigs has negative impacts on animal and carcass performance compared to other protein sources.

Finishing beef steers in the United States

According to USDA, cattle on feed are those which are fed a ration of grain, silage, hay, and/or protein supplements, for slaughter that are expected to grade USDA Select or better and excluding cattle being back-grounded for subsequent sale as feeder cattle (USDA-NASS, 2015). On January 1, 2015, in the United States, there were 10.7 million head of cattle on feed in feedlots with greater than 1000 head capacity compared to 10.1 million head in October 2014 (USDA-NASS, 2015; USDA-NASS, 2014). With a consistent 10 million head of cattle on feed, the feedlot sector of the beef industry has potential to provide a market of substantial size for PEAR.

According to Vasconcelos and Galyean (2007), a typical feedlot ration consists of 70-85% grain (corn), 8.3-9.0% roughage (corn silage or alfalfa), with the remainder of the ration consisting of plant based proteins (ethanol co-products), fat supplements (tallow), and minerals. A typical feedlot diet contains approximately 2.5 to 6.5% supplemental fat, often included to increase dietary energy density (Galyean and Gleghorn, 2001).

Grains including corn, sorghum, barley and wheat are included in high-concentrate diets, but in an attempt to increase digestibility, grain ingredients are typically further processed (Owens et al., 1997). To minimize impact on nutrient utilization from the addition of fat, feedlot diets should contain no more than approximately 6% supplemental fat (Hess et al., 2008). Care is also given to the amount of roughage in the diet, because roughage level has been shown to have impacts on not only finishing animal performance but also carcass characteristics because of effects on DM and NE intake (Galyean and Defoor, 2003).

Increased use of grains for ethanol production has resulted in the availability of grain co-products from dry and wet milling processes for use as protein and energy sources for ruminants. In a survey conducted in 2007, 82.8% of consulting feedlot nutritionists reported incorporating grain co-products in finishing rations with the average inclusion rate at approximately 16.5% (inclusion ranged from 5-50%; Vasconcelos and Galyean, 2007). Of the grain co-products used in finishing rations, wet and dry-distillers' grains as well as wet and dry corn gluten feed were among the most common.

Ethanol plants return nearly 30% of the initial corn DM used for ethanol to livestock production as distillers' grains (CAST, 2012). Distillers' grains are often included in rations as a lower cost protein or energy source and it has been suggested that PEAR is most likely to compete with or replace distillers' grains in feedlot rations (Drewery et al., 2012).

Protein and PEAR in finishing rations for beef steers

Crude protein in finishing rations for beef cattle can typically range from 12.5 to 14.4%, according to a survey of consulting nutritionists (Galyean, 1996). In a study by Thomson et al. (1995), where four different sources of supplemental protein (blood meal and corn gluten meal mix, cottonseed meal, soybean meal, or urea) were included in finishing rations at 11, 12, or 13% CP; animal performance was impacted, including daily gain, DMI, and gain:feed. Gain:feed increased linearly with increased CP level, while differences in carcass characteristics were minimal. Interpretation of the results of increasing protein level in the diet is difficult, especially in circumstances where energy intake also increases as a response to differential protein provision. Increases in gain in the previously described project could result from increased MP or NEg (NRC, 2000).

Post-extraction algal residue is expected to contain high levels of protein and as such would likely be treated as a protein source in beef cattle rations. Its high protein level, however, could be problematic when formulating rations, especially if used as a substitute for corn which is approximately 9.8% CP (DM basis; NRC, 2000). Over-provision of protein, compared to NRC (2000) recommendations provides a safety net for the variability reported in CP of by-products, including PEAR. Over fortification with protein can be used as a strategy to improve overall pen performance by providing additional protein to lighter and/or larger framed cattle that may be present in the pen when formulation was based on the average of cattle in the pen (Galyean, 1996), but benefits of doing so would need to outweigh costs.

The NRC (2000) was used to predict performance of finishing steers (453 kg BW) consuming PEAR included in a finishing ration, comprised of dry rolled corn, ground milo, cottonseed hulls, cottonseed meal, molasses, urea, and limestone. Assuming PEAR has a TDN value of 40% and the nutrient values reported by Drewery et al. (17.9% CP on a DM basis; 2014), average daily gain (with PEAR = 1.45 kg/d, without PEAR = 1.63 kg/d) and feed efficiency (predicted feed:gain with PEAR = 6.28:1 kg, without PEAR = 5.21:1 kg) are expected to be greater for steers not consuming PEAR. For predicting performance using the NRC model, corn was replaced with PEAR at an inclusion rate of 15%.

Fat content and fatty acid composition of beef

As previously mentioned, variability in processing methods used during lipid extraction from algal biomass alters the nutrient content of PEAR and potentially, its subsequent utilization. The resulting PEAR, after processing, is typically a fine, dusty feedstuff. Based solely on particle size it is anticipated that digestion of PEAR will primarily occur ruminally. In cattle diets, ruminal digestion typically increases as particle size decreases, and as a result, digestion in the small intestine increases as particle size increases (Owens et al., 1986). However, it is possible that some fraction of PEAR could bypass the rumen and be digested in the small intestine. If PEAR bypasses the rumen, the fatty acids present in the bypassed portion would not undergo ruminal biohydrogenation. Thus, fatty acids could then be absorbed in their original form, and would be represented as such in the resulting beef product, similar to monogastric absorption and representation of fatty acids in meat.

Ruminal biohydrogenation can also be bypassed if feed is covered in a material resistant to rumen microbial digestion (Ekeren et al., 1992); if processing of PEAR results in a substance resistant to digestion, PEAR could bypass the rumen. Additionally, if PEAR is produced that contains high levels of long chain omega-3 fatty acids, there could be potential for those fatty acids to bypass microbial biohydrogenation as well, however, results from previous studies are inconsistent (Ashes et al., 1992; Doreau and Chilliard, 1997; Gulati et al., 1999; Scollan et al., 2001; Shingfield et al., 2003; Lee et al., 2005). Therefore, there should be minimal expectation for beef from PEAR fed steers to contain high levels of EPA and DHA, even though algae is known to contain high levels, unless the fat portion of PEAR is enriched in EPA and DHA and PEAR bypasses the rumen and EPA and DHA are subsequently absorbed. However, other fatty acids present in PEAR could influence fatty acid content of beef from PEAR fed steers.

Fat content and fatty acid composition

Fat content and fatty acid composition of beef impacts its overall palatability (Mottram and Edwards, 1983; Savell and Cross, 1988). In the United States, the “window of acceptability” for fat in meat, as established by Savell and Cross (1988), is between 3 and 7.5% intramuscular lipid. In context, a muscle free of marbling, contains approximately 1% intramuscular lipid and as marbling increases; intramuscular lipid as a percentage increases. While percentage of intramuscular lipid is important and contributes to overall palatability of beef, it is arguable that type of intramuscular lipid plays just as important of a role concerning palatability. Individual fatty acids, which

collectively contribute to intramuscular lipid, have been shown to have direct effects on beef flavors (Larick et al., 1987).

As amount of marbling increases in beef, oleic acid content increases (Killinger et al., 2004; O'Quinn et al., 2012; Hunt et al., 2014). As oleic acid (**18:1**) has one double bond, it is considered a monounsaturated fatty acid (**MUFA**), just as other fatty acids containing one double bond are. In a study by May et al. (1993) almost half of consumers (47.5%) were able to detect differences in beef flavor between a high and low percentage MUFA beef product. In that particular study, beef from Angus and Wagyu cattle was compared for flavor differences. Of the two breeds, Wagyu beef was higher in 14:1, 16:1, and 18:1 suggesting that MUFA content of beef contributes to flavor.

Specifically, oleic acid content of subcutaneous fat, intramuscular fat, and *M. longissimus dorsi* samples was greater for Wagyu steers than Angus (45 vs. 50, 45 vs. 50, and 44 vs 45%, respectively), while amount of marbling was not different between breeds (May et al., 1993). While the May et al. (1993) study found that Wagyu beef higher in oleic acid and other PUFAs had a flavor difference to consumers, Rhee et al. (1990) also found that pork flavor was improved when oleic acid concentration was increased. Increasing levels of the fatty acids 16:0, 16:1cis9, and 18:1 were positively correlated to beefy/brothy and beef fat flavors, while increasing levels of the fatty acids 15:0, 18:3, 20:4, 20:5, 22:5, and 22:6 were negatively correlated (Larick et al., 1987).

Previously described evidence shows that flavor characteristics of meat have been shown to be dependent upon fatty acid composition; but, according to Shahidi and Rubin (1986), both genetics and environment play a critical role in flavor of red meats,

including beef, lamb, and pork. Species is stated to be the most important genetic factor, while feed source is the most significant environmental factor contributing to fat content and composition of beef. Even so, according to De Smet et al. (2004) fatty acid composition of meat is a result of a combination of factors in the live animal including primarily nutrition, but also genetics, fatness, and de novo synthesis of fatty acids. De novo synthesis of fatty acids can result from excess dietary glucose, acetate, and/or amino acids.

Pork is typically higher in linoleic acid than beef and lamb and consequently has a higher polyunsaturated: saturated fatty acid ratio (Calkins and Hodgen, 2007), however, beef and lamb typically has a higher n6:n3 fatty acid ratio (Wood and Enser, 1997). As supported by Shahidi and Rubin (1986) and Calkins and Hodgen (2007), changes in feed source or diet can alter fatty acid composition of meat. Specifically, Calkins and Hodgen (2007) suggest that feeding products higher in 18:3, 20:5, and 22:6 can increase n-6 fatty acids in pork, because polyunsaturated fatty acids undergo very little change during digestion in monogastrics.

Within species, it is known that feeds can impact the flavor of red meats, however, these effects have not been fully studied. According to Melton (1990), more research on the effects of feeds on red meat flavor could have the benefit of the production of leaner meat with more desirable flavor. Melton (1990) also hypothesized that certain feeds could be used in production to produce red meat products with specific flavors. Grass-fed beef tends to have associated grassy flavors, which are the result of particular fatty acids being deposited into the meat products (Larick and Turner, 1990;

Baublits et al., 2009). Beef cattle finished on grain or forage diets, with differing levels of 18:2 and 18:3 had respective differences in composition of volatile organic compounds, which is responsible for differences in flavor (Larick and Turner, 1990). Undesirable pork flavor can be the result of feeding products such as fish meal, spoiled meat scraps, horse manure, or cooked garbage during the last weeks prior to slaughter (Stringer, 1969). The impact of feed ingredient upon flavor source was dependent upon ingredient. Some feed sources impacted lipids, while others impacted lean muscle flavor (Stringer, 1969). While some feed ingredients influence sensory attributes of beef, not all feed ingredients do. According to Shand et al. (1998), feeding brewer's grains or wheat-based distillers' grains did not affect sensory characteristics of steaks compared to controls.

As flavor is a significant factor to consumers of beef, the beef industry developed a beef flavor lexicon to identify different beef flavor components (Adhikari et al., 2011). Miller and Kerth (2012) have done extensive work determining positive and negative flavors and their origin in lean or lipid portions of meat. Positive beef flavors include beefy, brown/roasted, bloody/serumy, fat-like, sweet, salty, and umami, which are all associated with lean portions of meat with the exception of fat-like. Negative beef flavors include metallic, liver-like, sour, barnyard, musty-earthly/humus, and bitter which are all associated with the lipid portion of meat (Miller and Kerth, 2012).

Altering fatty acid composition of beef through the manipulation of beef cattle feeding has the potential to be beneficial, but not just from effects on flavor. Flavor set aside, fatty acid composition of beef can impact health of consumers. Consuming five

ground beef patties for five weeks with high levels of 18:1 reduced risk factors of cardiovascular disease by increasing HDL-cholesterol in normocholesterolemic men and postmenopausal women, while LDL particle diameter was not reduced (Adams et al., 2010, Gilmore et al., 2011, 2013). Even though five patties were consumed each week for five weeks in each of the studies, and as a result, a large percentage of beef fat was consumed (ground beef was 20 - 34% fat, study dependent), it is important to recognize that no negative effects on cholesterol were observed, suggesting that fatty acid composition of beef has the potential to provide positive health benefits.

Marbling or intramuscular lipid

Marbling scores are used to assign USDA Quality Grades (**QG**), a driving factor in the value of a carcass and its associated cuts. In theory, QG is an indicative measure, but not a guarantee, of tenderness and palatability. A QG is assigned to an entire carcass, as an indicator of estimated quality in fabricated cuts. More specifically, individual cuts are not quality graded, but instead receive the QG assigned to the carcass. Quality grade is determined from carcass maturity and degree of marbling present in the *M.*

longissimus dorsi muscle at the 12th and 13th rib interface. Carcass maturity is scored based on ossification of the first three full thoracic buttons of the lumbar vertebra and lean color of the *M. longissimus dorsi* muscle at the 12th and 13th rib interface. Carcass maturity scores are based on the principal that meat from older animals is less tender.

Marbling scores can be used to predict tenderness and palatability. Marbling in top loin steaks, when evaluated by both consumer and trained sensory panelists, impacted eating quality of beef. Savell et al. (1987), conducted a study in three cities:

San Francisco, Philadelphia, and Kansas City using strip loins collected from 700 beef carcasses. They concluded, that of the factors measured, only marbling level and degree of doneness were significant drivers of eating quality. City, income, age, education level, and cooking method were not significant factors in consumer overall desirability ratings of steaks. Additionally, there was a significant city \times marbling level interaction for consumers in Philadelphia, but not in San Francisco or Kansas City, suggesting that consumers in different geographic regions could have stronger sensitivity to marbling level in beef strip steaks. In a well cited study by Smith et al. (1985), carcasses with higher QG produced more tender and palatable cuts, and there was less variability among higher QG. Small, but significant increases were found in juiciness, tenderness, and flavor as marbling score improved from practically devoid to moderately abundant. When comparing practically devoid and moderately abundant, steaks from the loin were more palatable 66% of the time and differences in marbling explained 33% of the variation in overall palatability, while those from the round were more palatable only 12.5% of the time and marbling explained 7% of the variation in overall palatability (Smith et al., 1985).

Marbling serves as protection for muscle fibers from overcooking. Overcooking can result in beef being less tender. According to Savell et al. (1987), steaks with higher degrees of marbling will still be juicy and tender when cooked to advanced degrees of doneness while those with lower degrees of marbling will be dry and tough. Marbling contributes to tenderness in several different ways according to Miller (1994), who suggests the following theories. Fat can act as a lubricant as it is melted during the

cooking process. The oils, or melted fat, that result from cooking essentially work as a lubricant between the teeth and mouth during chewing. Additionally, intramuscular fat can create gaps between muscle fibers, making the muscle less dense, and thus more tender after cooking. As fat melts, it leaves empty pockets, decreasing density, therefore requiring less force to chew or shear through a meat sample.

Beef consumers prefer meat that is reasonably marbled and juicy, and it is a realistic goal for beef producers to provide what consumers desire to purchase. In order to appropriately produce a product that consumers want, it is important to understand the mechanisms by which marbling and juiciness are impacted. In a recent study, it was determined through the use of a consumer panel that consumers rated *longissimus* muscle steaks more favorable when having a higher fat content (Hunt et al., 2014).

According to the conclusions of most recent National Beef Quality Audit, conducted in 2011, the beef industry is making progress towards providing a more uniform, consistent product to United States beef consumers. Greater than 58% of carcasses graded USDA Choice while other QG of Prime, Select, Standard, Commercial and Utility grades were found to be 2.1, 32.6, 5.1, 0.9, and 0.3%, respectively (Moore et al., 2012).

Beef tenderness

Warner-Bratzler Shear Force (**WBSF**) is the most commonly used and accepted measurement of tenderness. Warner-Bratzler Shear Force measures the force required to shear through a cored meat sample, which simulates chewing. To be considered “very tender,” shear force values need to measure less than 3.2 kg, “tender,” greater than 3.2

kg but less than 3.9 kg, “intermediate,” greater than 3.9 kg but less than 4.6 kg and, “tough,” greater than 4.6 kg (Destefanis, et al., 2008). This WBSF threshold value for tough is similar to that of Shackelford et al. (1999) who found that shear force values greater than 4.6 kg are indicative of “tough” steaks.

According to the 1991 National Beef Tenderness Survey, Morgan et al. (1991) found that 23% of cuts from the rib and 18% of cuts from the loin had shear force values greater than 3.9 kg. More recently, Guelker et al. (2013) found that WBSF values for steaks from the chuck, rib, and loin found in retail stores, which were surveyed for the 2010 National Beef Tenderness Survey, were mostly “very tender.” Of steaks surveyed, 96-100% of top blade, boneless ribeye, bone in ribeye, boneless top loin, t-bone, porterhouse, top sirloin steaks; 87-89% top loin and top round steaks; and 71% of bottom round steaks were rated as “tender” or higher according to WBSF values as outlined by Destefanis et al. (2008) and Guelker et al. (2013). Of the ten types of steaks surveyed, only top loin (2.17%), bone in top loin (4.35%), top round (4.35%), and bottom round (5.26%) steaks were found to have “Tough” WBSF scores (Guelker et al., 2013). Results of Guelker et al. (2013) suggest that tenderness is not currently an issue for beef steaks produced in the United States, except for those steaks from the round, which have more variability in tenderness than steaks from the other primals.

Color and label information effect flavor perception

Consumers mis-identify flavor differences when provided label information related to added ingredients or processing. In a study using candy M&Ms of different colors and M&Ms provided label information “dark chocolate” or “milk chocolate”,

consumers identified differences in flavor when the product being tested was the same (Shankar et al., 2009). Consumers ranked the “dark chocolate” labeled M&Ms as significantly more *chocolatey* than “milk chocolate” labeled M&Ms and ranked brown M&Ms as being significantly more *chocolatey* than green colored M&Ms (Shankar et al., 2009). Using the M&M study as an example, one challenge from feeding PEAR could be the perceived flavor differences resulting from labeling or rumors. When consumers learn that cattle are fed algae or algae co-product, this information may create a negative perception of product flavor (if algae is considered bad, as in pond scum), or a more positive perception (if algal fuels are considered ‘green’ or socially redeeming in some manner).

Without considering price, consumers use three sensory properties to evaluate meat quality when making purchasing decisions: appearance, texture, and flavor. Of appearance, texture, and flavor, visual appearance of meat is the most important factor because it most influences initial purchasing decisions (Kropf et al., 1986; Faustman and Cassens, 1990). Meat color is the most important visual attribute. Consumers view discoloration as a sign of unwholesomeness and discriminate against cuts with discoloration because the cuts lack a fresh appearance (Kropf et al., 1986). In grocery stores today, cuts of meat that become discolored are marketed at reduced prices, aimed to target more price than quality conscious consumers. In contrast, when evaluating an eating experience rather than a purchasing decision, studies have shown that tenderness and flavor are the most important attributes of beef to consumers (Neely et al., 1998; Goodson et al., 2012).

Strip steak color was redder when steers were fed a combination of condensed distillers' solubles and barley, when compared to strip steaks from steers fed corn gluten feed (Dahlen et. al, 2001). Also, Roeber et al. (2005) found that including distiller's grains in finishing diets could have a negative impact on color stability during retail display, when fed at rates exceeding 40-50% dietary DM. However, when fed at rates between 10-25% dietary DM, color stability during shelf life is maintained and even enhanced in some cases. Additionally, the inclusion of distillers' grains did not affect cooked beef palatability. Therefore, the effect of the by-product, PEAR, on beef shelf life and palatability should be investigated.

An example of a feed ingredient included in beef cattle rations that impacts beef quality is Vitamin E. In a study by Williams et al. (1992), beef cuts from Holstein steers supplemented with Vitamin E were shipped to retail stores in three U.S. cities. While quantity of meat sold was the same over the 4 d period, less product from steers fed Vitamin E was discounted due to discoloration allowing for greater profit margin. While supplementation of Vitamin E provides retailers beef with a longer shelf-life, the cost of supplementation is assigned to cattle feeders. Therefore, an appropriate incentive for cattle feeders to incur the additional expense needs to be developed. Cost of supplementation of Vitamin E was estimated to be \$3 per animal (Liu et al., 1995).

Producers are hesitant to incur \$3 per animal cost of Vitamin E, when the benefit goes to the retailer of beef. Therefore, it is difficult to persuade to feed or exclude ingredients based on beef quality with the continued absence of either incentives or penalties.

Conclusion

Changes in population and an expected increase in demand for high quality beef makes it necessary to evaluate alternative feed sources for feeding beef cattle. Utilizing PEAR, a co-product of biofuel production from micro-algae, as a feedstuff for cattle creates a market for a by-product potentially produced in large quantities and provides a non-competitive source of nutrients for beef cattle. The protein content and fatty acid composition of PEAR, suggests PEAR would be best utilized as a protein source in finishing rations of beef steers. A series of projects will need to be conducted to determine if PEAR can be successfully incorporated into finishing rations of beef steers. The following objectives were evaluated in a series of three projects: 1) compare the effects of PEAR on intake and nutrient utilization versus glucose infused ruminally or post-ruminally, 2) determine effects of PEAR supplementation on carcass performance, 3) evaluate the effects on flavor and tenderness of beef strip steaks using an expert-trained sensory panel and WBSF, 4) determine ability of consumer panelists to detect difference in ground round and ground chuck flavor from steers fed PEAR versus those infused with glucose ruminally. Understanding the effects of PEAR inclusion in beef cattle diets on nutrient utilization and beef quality and flavor will enable recommendations for its effective utilization in beef production systems.

CHAPTER II

EFFECT OF INCLUSION OF POST-EXTRACTION ALGAL RESIDUE ON NUTRIENT UTILIZATION AND CARCASS PERFORMANCE IN FINISHING STEERS

Overview

An experiment was conducted to determine effects of post-extraction algal residue (**PEAR**) inclusion on nutrient utilization and carcass characteristics in finishing steers. Eighteen Angus × Hereford steers (initial BW = 549 ± 38.8 kg) were randomly assigned to one of three treatments: PEAR hand-mixed into the diet at 1.0 kg OM/d (**PEAR**), 1.0 kg OM/d glucose infused ruminally (**GR**) or abomasally (**GA**). Infused steers were ruminally cannulated, allowing continuous infusion of glucose via anchored infusion lines. Basal diets consisted of dry rolled corn (42.3%), ground milo (18.0%), cottonseed hulls (13.5%), grass hay (10.0%), molasses (6.7%), cottonseed meal (5.4%), vitamin/mineral premix (2.3%), urea (0.9%), and limestone (0.9%). Steers were adapted to housing and basal diet for 5 d; subsequently, treatments were applied for 35 d, until harvest. Intake was measured daily and digestion was determined from d 27 to 31 of treatment application using fecal grab samples. Forty-eight h post-harvest, carcass measurements were collected from each carcass. Greater DMI was observed for PEAR (13.0 kg/d) than GR (10.3 kg/d; $P < 0.05$); DMI for steers receiving GA (11.2 kg/d) was intermediate and not different from either PEAR or GR ($P \geq 0.14$). Intake of DE was similar among treatments ($P = 0.45$) and averaged 36 Mcal/d as was digestible OM

intake and averaged 8.8 kg/d ($P = 0.51$). Digestion of GE was 72.9, 82.6, and 80.9% for PEAR, GA, and GR, respectively ($P < 0.01$). Digestion of NDF was substantially less (55.7%) for PEAR than GA (75.4%) and GR (75.0%; $P < 0.01$). Steers fed PEAR had greater marbling scores (Mt^{20}) than GA (Sm^{63}) and GR (Sm^{52} ; $P = 0.01$). Accordingly, USDA Quality Grade was greater for PEAR than GA and GR ($P = 0.01$; Ch^{40} , Ch^{21} , and Ch^{17} , respectively). There was no difference in USDA Yield Grade or HCW between treatments ($P \geq 0.66$). Diet digestibility was impacted and carcass quality was slightly improved by inclusion of PEAR in the diet of finishing steers; however, further investigation is necessary to determine consumer acceptance of beef from PEAR-fed steers.

Introduction

The inclusion of cottonseed meal (**CSM**), soybean meal (**SBM**), and distillers' grains (**DG**) demonstrates the ability to include co-products as sources of protein or energy in cattle rations. Post-extraction algal residue (**PEAR**), a co-product which originates from biofuel production from algal biomass, is a potential feedstuff for beef cattle.

In its current state, biofuel production from micro-algae fails to be cost-competitive with other fuel sources (Bryant et al., 2012). However, market development for PEAR would aid in cost recovery, allowing biofuel from micro-algae to be more cost-competitive with other oils and viable as an industry. After oil is extracted from algae, more of the original biomass remains as PEAR than was removed as oil (>50% of biomass; Christi, 2007) so a substantial amount of PEAR would be available. With 10.7 million cattle on feed (USDA-NASS, 2015), the U.S. feedlot sector is an appealing market of substantial size for PEAR. Placement of DG in finishing rations demonstrates an acceptance of a competitive biofuel co-product and provides a model of nutrient cycling that enhances both biofuel and beef sustainability.

Drewery (2012) showed that cattle consumed and utilized PEAR in a similar manner to conventional protein supplements such as CSM, SBM, and DG. Since the completion of the Drewery (2012) work with a first-generation PEAR, a second-generation PEAR has been produced. Second-generation PEAR is greater in nutritional value as it contains less ash (12.2 vs 45.5%) and more protein (33.8 vs 17.9% CP; Drewery et al., 2014; Table 1). These changes in nutrient composition have come as

methods for growing, harvesting, and extracting algae have improved. However, PEAR has not, to our knowledge, been extensively evaluated as a component of finishing rations.

Accordingly, our objectives were to evaluate the effects of PEAR provision on nutrient utilization of cattle consuming finishing rations as well as its impact on carcass performance.

Materials and methods

Eighteen Angus × Hereford steers (initial BW = 549 ± 38.8 kg) were used in a one-way, completely randomized, three-treatment experiment designed to evaluate the effects of PEAR on nutrient utilization as compared to infusion of glucose ruminally or abomasally. Treatments included PEAR hand mixed into the diet (1.0 kg OM/d; PEAR), and ruminal (GR), or abomasal (GA) infusion of 1.0 kg OM/d glucose. Algal biomass (*Chlorella* sp.) was grown photosynthetically in an open pond, flocculated, dewatered, spray dried, and then extracted with a methylpentane solvent to produce the PEAR used in this experiment. Infused steers were ruminally cannulated, allowing continuous infusion of glucose via anchored infusion lines. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC 2014-0003) at Texas A&M University.

Table 1. Chemical composition of finishing ration and post-extraction algal residue (PEAR)¹.

Item	Finishing Ration	PEAR ¹
DM, %	92.6	93.3
	----- % of Dry Matter -----	
OM	94.3	87.8
CP	13.7	33.8
Ether extract	3.40	3.91
Acid hydrolysis fat	3.97	6.13
ADF	17.40	n.d.
NDF	33.57	n.d.
Macrominerals, %		
Ca	1.31	0.08
P	0.35	0.54
K	0.93	0.64
Mg	0.23	0.09
Na	0.30	3.16
S	0.21	0.74
Microminerals, ppm		
Al	121.6	2880.0
Co	0.83	0.83
Cu	15.85	54.70
Fe	165	3540
Mn	84.3	61.1
Mb	0.89	0.88
Zn	98.9	164.0

¹PEAR = post-extraction algal residue (*Chlorella* sp.)

Steers were housed in continuously lighted barns in individual pens and steers were provided *ad libitum* access to fresh water. Additionally, steers were provided a

finishing ration as a basal diet at 130% voluntary intake for the duration of the study (Tables 1 and 2). The finishing ration was comprised of dry rolled corn (42.3%), ground milo (18.0%), cottonseed hulls (13.5%), grass hay (10.0%), molasses (6.7%), cottonseed meal (5.4%), vitamin/mineral premix (2.3%), urea (0.9%), and limestone (0.9%).

Table 2. Comparison of fatty acid composition of finishing ration and post-extraction algal residue (PEAR)¹.

Item	Finishing Ration	PEAR ¹
Fatty Acid, g/100g FAME ²		
14:0	0.26	0.72
14:1	0.05	0.41
16:0	17.32	26.19
16:1	0.29	1.99
18:0	2.36	4.12
18:1c9	21.30	37.89
18:1c11	0.65	5.32
18:2	48.63	4.47
18:3	4.85	5.03
20:0	0.03	n.d. ³
20:1	0.14	0.62
20:2	0.03	0.32
20:4	0.27	0.28
20:5	0.26	0.36
22:0	0.32	0.84
24:0	0.10	n.d.
22:6	0.10	0.11

¹PEAR = post-extraction algal residue (*Chlorella* sp.)

²FAME = fatty acid methyl esters

³n.d. = not detected

Prior to applying treatments, steers were adapted to the barn, diet, and feeding protocols for 5 d. Throughout the subsequent 35 d feeding period steers received their respective treatments. For the first 3 d, treatments were administered at increasing levels (0.25 kg OM/d increments) to prevent sudden intake changes. Sampling for nutrient utilization took place on d 27 through d 31. At the end of the nutrient utilization sampling period, harvesting of steers began on d 34 and continued through d 36. Harvest day was assigned at random with respect to treatment and rumen cannulation. On the day of harvest, steers were transported approximately 9 km to the Texas A&M University Rosenthal Meat Science & Technology Center, where the cattle were harvested by humane, industry standard procedures. Prior to harvest, steers with ruminal cannulae were ruminally evacuated to prevent carcass contamination during harvest; PEAR steers were fasted for 18 h prior to slaughter. Carcasses were evaluated for USDA Quality and Yield Grade data 48 h post slaughter according to USDA standards (USDA, 1997).

Steers receiving infusion treatments (GA and GR) received their treatments and were harvested in two randomly assigned periods, due to capacity for administering treatments and harvesting. The first period was conducted from December 2013 – January 2014 and the second period was conducted from January 2014 – February 2014. During data analysis, period was not used to account for error as the PEAR treatment was not administered during the first period.

Administration of treatments were as follows: 1.0 kg/d OM PEAR hand mixed into diet daily at 0600 h or 1.0 kg OM of glucose continuously infused through an anchored infusion line into the rumen or abomasum.

Intake was measured daily, however, calculations of intake were made from observations on d 27 through d 30. Hay, finishing ration, supplement (PEAR and glucose), and ort samples were collected on d 27 through d 30 to correspond with fecal samples collected d 28 through d 31 for determination of digestion. Feed refusals were collected and weighed prior to feeding at 0600 h. A 200 g sample of orts was retained for analysis. Fecal production was estimated using titanium dioxide as an internal marker. Titanium dioxide (10 g/d) was hand mixed into the diet prior to feeding on d 21 through d 31 to estimate fecal output. One d prior to feeding titanium (d 20), a fecal sample was collected, to determine baseline titanium levels. On d 28 through d 31 fecal samples were collected every 8 h with sample time advancing 2 h each day so that 12 samples were obtained over a 4 d collection period. Samples collected during the feeding of titanium were composited then frozen at -20°C upon collection until analysis. Prior to analysis, samples were allowed to thaw and were then thoroughly mixed. A representative subsample was collected and used for analysis.

Laboratory analyses

Hay, orts, finishing ration, PEAR, and fecal samples were dried in a forced-air oven for 96 h at 55°C and allowed to air-equilibrate then weighed for determination of partial DM. Hay, mixed ration, and PEAR samples were pooled across days on an equal weight basis. Ort samples were composited by steer across days. Hay, finishing ration, orts, and fecal samples were then ground through a 1-mm screen using a Wiley mill and dried at 105°C for determination of DM. Organic matter was determined as the loss in dry weight upon combustion in a muffle furnace for 8 h at 450°C. Nitrogen was

measured using the Elementar rapid N cube (Elementar, Hanua, Germany) and CP was calculated as $N \times 6.25$ (for feed and hay only). Analysis for NDF and ADF was performed sequentially using an Ankom Fiber Analyzer with sodium sulfite omitted and without correction for residual ash (Ankom Technology Corp., Macedon, NY). Physical structure and particle size of PEAR and glucose prevented determinations of NDF and ADF for those samples.

Total lipids of finishing ration, hay, and PEAR were extracted by a modification of the method of Folch et al. (1957). One hundred milligrams of each sample were extracted in chloroform:methanol (2:1, v/v) and fatty acid methyl esters (**FAME**) were prepared as described by Morrison and Smith (1964), modified to include an additional saponification step (Archibeque et al., 2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m \times 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with hydrogen as the carrier gas (flow rate = 35 mL/min; split ratio 20:1). Initial oven temperature was 150°C; oven temperature was increased at 5°C/min to 220°C and held for 22 min. Total run time was 52 min. Injector and detector temperatures were at 270°C and 300°C, respectively. Individual fatty acids were identified using genuine external standard GLC-68D (Nu-Chek Prep, Inc., Elysian, MN).

Samples were also sent to SDK Labs (Hutchinson, KS) for analysis of ether extract, acid hydrolysis fat, mineral composition, and heavy metal analysis. Additionally, a PEAR sample was sent to SDK Labs for CP analysis.

Calculations

Titanium dioxide was used as a marker to estimate fecal production for calculation of digestion, using methods as described by Cochran and Galyean (1994).

Statistical analyses

Intake, digestion, and carcass performance traits were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The only term in the model was treatment. The LSMEANS option was used to calculate treatment means and all-pair wise comparisons were included as follows: GA vs. GR, GA vs. PEAR, and GR vs. PEAR. A treatment difference was considered significant if $P \leq 0.1$. A pairwise comparison difference was considered significant if $P \leq 0.05$.

Results

Greater DMI was observed for PEAR (13.0 kg/d) than GR (10.3 kg/d; $P < 0.05$); DMI for steers receiving GA (11.2 kg/d) was intermediate and not different from either PEAR or GR ($P = 0.14$; Table 3). Dietary energy density (DE, ME, NEm, and NEg Mcal/kg), was lower for PEAR compared to GA and GR ($P \leq 0.01$), but was similar between GA and GR ($P \geq 0.05$). Intake of OM, digestible OM, NDF, and ADF was not significantly different between treatments, ($P > 0.10$). Additionally, intake of DE (**DEI**) was not different ($P = 0.45$) and averaged 36 Mcal/d.

Table 3. Nutrient utilization of beef steers consuming post-extraction algal residue (PEAR)¹ or receiving glucose infusion².

Item	Treatments ³			SEM	P-value
	GA	GR	PEAR		
Intake, kg/d					
DM	11.19 ^{a,b}	10.30 ^a	13.01 ^b	0.83	0.09
OM	10.56	9.75	12.21	0.78	0.11
Digestible OM	9.12	8.19	9.03	0.61	0.51
NDF	3.52	3.25	3.98	0.29	0.24
ADF	1.92	1.76	2.05	0.17	0.47
DEI, Mcal/d	37.47	33.89	38.31	2.57	0.45
Energy Content ⁴ , Mcal/kg					
DE	3.34 ^a	3.31 ^a	2.95 ^b	0.05	< 0.01
ME	2.74 ^a	2.71 ^a	2.42 ^b	0.04	< 0.01
NEm	1.81 ^a	1.79 ^a	1.54 ^b	0.04	< 0.01
NEg	1.18 ^a	1.16 ^a	0.94 ^b	0.03	< 0.01
Total Tract Digestibility, %					
DM	84.9 ^a	83.4 ^a	73.2 ^b	1.2	< 0.01
OM	85.9 ^a	84.5 ^a	74.1 ^b	1.2	< 0.01
NDF	75.4 ^a	75.0 ^a	55.7 ^b	2.2	< 0.01
ADF	66.5 ^a	67.5 ^a	34.7 ^b	3.4	< 0.01
GE	82.6 ^a	80.9 ^a	72.9 ^b	1.4	< 0.01

¹PEAR = post extraction algal residue (*Chlorella* sp.)

²Within each row, means with differing subscripts differ ($P \leq 0.05$)

³GA = 1.0 kg OM glucose infused into the abomasum daily; GR = 1.0 kg OM glucose infused into the rumen daily; PEAR = 1 kg OM post-extraction algal residue fed daily

⁴ME, NEm, NEg were calculated using the Beef Cattle NRC (2000)

Digestion of DM, OM, NFD, ADF, and GE was significantly lower for PEAR compared to GA and GR ($P < 0.01$), but not significantly different between GA and GR ($P > 0.05$). Digestion of GE was lower ($P \leq 0.05$) for PEAR than GA and GR 72.9%, 82.6, and 80.9% for PEAR, GA, and GR, respectively ($P \leq 0.01$). Digestion of NDF was substantially less (55.7%) for PEAR than GA (75.4%) and GR (75.0%; $P < 0.01$).

Intake of minerals (Na, Al, Cu, and Fe) was greater ($P < 0.01$) for PEAR compared to GA and GR (Table 4). Specifically, intake of Na was approximately 2.5 times higher for PEAR ($P \leq 0.05$) than GA and GR. Fatty acid intake (16:0, 18:0, 18:1, 18:2, 18:3, 20:5, and 22:6) was also greatest for PEAR. Intake of 16:0, 18:0, 18:1, 18:3, 20:5, and 22:6 was significantly greater for PEAR compared to GA and GR ($P < 0.01$), but was not statistically different between GA and GR ($P > 0.05$). Additionally, intake of 18:2 was greatest overall and averaged 168 g/d; but was only different between GR and PEAR ($P < 0.01$).

Table 4. Mineral and fatty acid intake of beef steers consuming post-extraction algal residue (PEAR)¹ or receiving glucose infusion².

	Treatments ³				
Item	GA	GR	PEAR	SEM	P-value
Minerals					
Na, g/d	30.58 ^a	28.06 ^a	70.35 ^b	2.56	< 0.01
Al, mg/d	1235 ^a	1133 ^a	4635 ^b	109.7	< 0.01
Cu, mg/d	160 ^a	147 ^a	249 ^b	13.1	< 0.01
Fe, mg/d	1679 ^a	1540 ^a	5883 ^b	147.5	< 0.01
Fatty acids, g/d					
16:0	53.63 ^a	49.20 ^a	73.34 ^b	4.35	< 0.01
18:0	7.49 ^a	6.87 ^a	10.43 ^b	0.61	< 0.01
18:1	71.74 ^a	65.81 ^a	99.50 ^b	5.82	< 0.01
18:2	162.64 ^{a,b}	149.20 ^a	193.04 ^b	13.12	0.08
18:3	6.13 ^a	5.63 ^a	9.18 ^b	0.71	< 0.01
20:5	0.73 ^a	0.67 ^a	1.00 ^b	0.05	< 0.01
22:6	0.20 ^a	0.18 ^a	0.28 ^b	0.02	< 0.01

¹PEAR = post extraction algal residue (*Chlorella* sp.)

²Within each row, means with differing subscripts differ ($P \leq 0.05$)

³GA = 1.0 kg OM glucose infused into the abomasum daily; GR = 1.0 kg OM glucose infused into the rumen daily; PEAR = 1 kg OM post-extraction algal residue fed daily

There was no difference in USDA Yield Grade or corresponding factors used to determine USDA Yield Grade (HCW, fat thickness, adjusted fat thickness, LM area, or KPH) between treatments ($P > 0.58$; Table 5). Steers fed PEAR had greater marbling scores (520) than GA (463) and GR (452; $P = 0.01$). Accordingly, USDA Quality Grade was greater for PEAR than GA and GR ($P = 0.01$; 340, 321, and 317, respectively).

Table 5. Carcass traits for steers consuming post-extraction algal residue (PEAR)¹ or receiving glucose infusion².

Item	Treatments ³			SEM	P-value
	GA	GR	PEAR		
HCW, kg	353	342	341	10	0.66
Fat thickness, cm	2.18	2.26	2.29	0.21	0.93
Adjusted fat thickness, cm	2.43	2.56	2.50	0.20	0.91
LM area, cm ²	71.8	70.3	71.6	2.0	0.84
Internal fat (KPH), %	2.00	2.08	2.25	0.17	0.58
Yield grade	4.69	4.81	4.72	0.25	0.93
Quality grade ⁴	321 ^a	317 ^a	340 ^b	5	0.01
Marbling score ⁵	463 ^a	452 ^a	520 ^b	15	0.01

¹PEAR = post extraction algal residue (*Chlorella* sp.)

²Within each row, means with differing subscripts differ ($P \leq 0.05$)

³GA = 1.0 kg OM glucose infused into the abomasum daily; GR = 1.0 kg OM glucose infused into the rumen daily; PEAR = 1 kg OM post-extraction algal residue fed daily

⁴Quality grade: 300 = USDA Choice

⁵Marbling score: 400 = Small⁰⁰; 500 = Modest⁰⁰

Discussion

This study was performed to evaluate the effects of PEAR inclusion on nutrient utilization and carcass characteristics of steers consuming a finishing ration. While provision of PEAR increased DMI, it did not result in differences in OM intake due to the high ash content (12%) of PEAR and the delivery of treatments on an equal OM

basis. Treatments were designed to determine how replacing OM from glucose with PEAR impacts nutrient utilization and carcass characteristics. Increased DMI with inclusion of PEAR was similar to results found by Brauer et al. (2014); which observed that as a PEAR supplement was included in a corn based diet at 10% (similar to our inclusion rate), DMI numerically increased from 6.9 to 8.0 kg/d when comparing controls to PEAR fed steers. Contrarily, Brauer et al. (2014) also observed that when PEAR inclusion was increased to 15% of the diet with and without supplemental urea, there was not a difference in DMI compared to control steers (6.2, 6.7, and 6.8 kg/d, respectively). When evaluating straw intake with supplemental first generation PEAR, Drewery et al. (2014) found that PEAR increased straw OM intake from 1.92 kg/d for controls to as much as 2.78 kg/d with the highest inclusion rate for PEAR in the study ($P = 0.05$), which is consistent with previous studies where supplementing protein to steers consuming low-quality forage increased intake (Köster et al., 1996; Mathis et al., 2000; Wickersham et al., 2004).

Provision of PEAR decreased digestibility of the diet compared to glucose, with NDF and ADF digestion being the most impacted. Post-extraction algal residue is a source of protein (33.8% CP), fat (3.9% EE), and Na (3.2%), but also contains 12.2% ash; PEAR is not expected to be a source of fiber as measured by NDF and ADF. As such, no measurable indigestible NDF was added to the finishing diet by inclusion of PEAR; thus, effects on NDF digestion should be attributed to an alternative mechanism. We suggest that the decreased digestibility of nutrients was likely caused by the higher ash content of PEAR compared to glucose. Additionally, digestibility could have been

impacted as a result of increased rate of passage due to increased DMI (thus, greater digesta volume) or increased Na consumption (thus, greater water intake, as PEAR contains 3.2% Na) with PEAR inclusion. In a study by Berger et al. (1980), as NaOH was added in the diet, rate of passage increased, likely due to stimulated increased water intake, resulting in less viscous digesta. Furthermore, the infused glucose, which was dissolved in water, was administered continuously through infusion lines. Thus, the glucose treatments did not contribute to the volume of digesta in the rumen. In a study performed by Schettini et al. (1999), tennis balls were added to the rumen of cannulated cows and rate of passage was measured. As tennis balls were added, rate of passage increased, suggesting that mass and volume of rumen content alters rate of passage ($P < 0.05$). Therefore, if rate of passage was altered due to viscosity or volume of digesta, digestibility could also have been impacted.

When Drewery et al. (2014) included PEAR in diets of steers consuming wheat straw total tract NDF digestion responded quadratically with increasing levels of PEAR in the diet ($P < 0.01$). When PEAR was included at 50, 100 and 150 mg N/kg, total tract NDF digestion was 55.8, 50.2, and 44.2%, respectively, compared to 49.7% for controls steers (Drewery et al., 2014). Ruminal NDF digestion was not impacted with PEAR inclusion ($P = 0.42$; Drewery et al., 2014). As total tract digestibility decreased with increased level of PEAR or mg N/kg, it could be hypothesized that excess or added CP (thus N), resulting in increased intake, in PEAR steers compared to GA and GR could have resulted in decreased NDF digestibility, comparatively.

Results of previous studies have shown that inclusion of lipid extracted algae supplements similar to PEAR have had effects on acetate and propionate production and acetate:propionate ratios (Beckman et al., 2012; Drewery et al., 2014; Lodge-Ivey et al., 2014). In a study utilizing lambs, Beckman et al. (2012) observed that inclusion of an algae supplement numerically decreased acetate and significantly increased ruminal propionate ($P \leq 0.05$) compared to supplemental hay and decreased the acetate:propionate ratio from 5 to 3.7 ($P \leq 0.05$). In steers, Drewery et al. (2014) found that as provision of PEAR increased, acetate decreased linearly ($P = 0.04$) and propionate numerically increased ($P = 0.22$). In an in-vitro study conducted using rumen fluid, the acetate:propionate ratio decreased from 2.4 to an average ratio of 1.4 for three different types of lipid extracted algae supplement of the *Chlorella* sp. ($P \leq 0.05$; Lodge-Ivey et al., 2014). Those steers receiving PEAR in our study were not ruminally cannulated, so no information concerning the production or concentration of ruminal VFA is available; however, it is hypothesized that inclusion of PEAR resulted in a similar response to VFA production in the rumen found by Beckman et al. (2012), Drewery et al. (2014), and Lodge-Ivey et al. (2014) who used a PEAR type supplement similar to our PEAR. As propionate is likely to have increased and the corresponding acetate:propionate ratio is likely to have decreased, it is possible for additional propionate (a potential gluconeogenic, marbling precursor) to have been available in steers consuming PEAR.

Research from Smith and Crouse (1984) suggests intramuscular adipose cells primarily derive acetyl units for lipid synthesis from glucose, while subcutaneous

adipose tissue primarily uses acetate for lipid synthesis. Specifically, in an *in vitro* study using adipose tissue from Angus steers, glucose provided 1-10% while acetate provided 70-80% of the acetyl units for lipid synthesis in subcutaneous adipose tissue while glucose provided 50-75% and acetate provided 10-25% of the acetyl units for lipid synthesis in intramuscular adipose tissue (Smith and Crouse, 1984). However, as the number of adipocytes increases, the contribution of glucose to lipid synthesis decreases, therefore, efficiency and utilization of glucose to promote marbling decreases. As feeder cattle typically fatten with time on feed, thus age, marbling development would be expected to be deposited at a faster rate in younger feeder cattle compared to older feeder cattle. In reference to the YG data, subcutaneous fat thickness was not different ($P = 0.93$) among treatments, so the utilization of glucose for lipid synthesis should have been similar and marbling deposited at similar rates, unless there were additional marbling precursors provided in the diet from PEAR, such as increased propionate from PEAR inclusion.

Marbling, which is a determining factor for USDA Quality Grade contributes to the overall value of a beef carcass. Higher degrees of marbling result in higher price premiums for beef. Marbling contributes to palatability characteristics, including juiciness, tenderness, and flavor. As marbling increases in beef, oleic acid (18:1n-9) content typically increases (Killinger et al., 2004; O'Quinn et al., 2012; Hunt et al., 2014). An increase in oleic acid, a MUFA, is desirable because higher levels of MUFA in beef positively influence beef palatability (Waldman et al., 1968; Westerling and

Hedrick, 1979). Additionally, beef high in oleic acid has been shown to reduce risk factors for cardiovascular disease (Adams et al., 2010; Gilmore et al., 2011, 2013).

Another notable observation from the data is that intake values for DM, OM, NDF, ADF, and DE for GA were closer to values observed for PEAR than were values observed for GR. Other values that follow this similar pattern are USDA Quality Grade and marbling score. This phenomenon could suggest that PEAR nutrient intake and utilization was more similar to GA intake and utilization, which could be related to marbling score, and correspondingly USDA Quality Grade.

Conclusion

The purpose of this experiment was to: 1) determine the impact of PEAR on nutrient utilization and 2) compare the impacts of treatments on carcass characteristics. The inclusion of PEAR caused digestibility of the diet to decrease. The results of this study suggest that PEAR could be included at the rate of 1.0 kg/d OM to provide increased USDA Quality Grade, an important carcass value determining characteristic without decreasing intake and alternating USDA Yield Grade. The optimum inclusion level of PEAR for impact on nutrient utilization and carcass value determining characteristics, however should be further investigated. Furthermore, the impact of PEAR on palatability of beef and consumer acceptance of beef from PEAR or algae-fed steers should be studied.

CHAPTER III

EFFECT OF INCLUSION OF POST-EXTRACTION ALGAL RESIDUE IN FINISHING RATIONS OF BEEF STEERS ON STRIP STEAK AND GROUND BEEF FLAVOR

Overview

Microalgae cultivation as a biofuel source will yield a high volume of post-extraction algal residue (**PEAR**) that could be fed to ruminants. Inclusion of PEAR in finishing diets of beef cattle is only viable if it does not have negative effects on beef quality and flavor. In a three phase experiment, 18 Angus × Hereford steers (BW = 549 ± 38.8 kg) were randomly assigned to one of three treatments: PEAR hand-mixed into the diet at 1.0 kg OM/d (PEAR), 1.0 kg OM/d glucose infused ruminally (**GR**) or abomasally (**GA**). Infused steers were ruminally cannulated, facilitating continuous infusion of glucose via anchored infusion lines. Basal diets consisted of dry rolled corn (42.3%), ground milo (18.0%), cottonseed hulls (13.5%), grass hay (10.0%) molasses (6.7%), cottonseed meal (5.4%), vitamin/mineral premix (2.3%), urea (0.9%), and limestone (0.9%). Steers were adapted to housing and basal diet for 5 d; subsequently, treatments were applied for 35 d, until harvest. Forty-eight h post-harvest, strip steaks were collected from each carcass from GA, GR, and PEAR treatments for analysis by an expert trained sensory panel in Phase 1. Additionally, at 48 h post-harvest strip steaks were collected from each carcass from GR and PEAR treatments for tenderness evaluation in Phase 2. Seventy-two h post-harvest, beef subprimals and subcutaneous

adipose tissue from the chuck and round of each carcass from GR and PEAR treatments were collected for evaluation by a consumer panel in Phase 3. Data from Phase 3 were analyzed as a 2×2 factorial treatment arrangement (diet: GR or PEAR; primal: chuck or round). No off-flavors were detected by trained sensory panel analysis in strip steaks from GA, GR, or PEAR ($P > 0.05$). No significant differences for *overall*, *overall flavor*, *beef flavor*, or *juiciness liking* were observed in ground round or ground chuck from PEAR or GR fed steers ($P \geq 0.17$). Inclusion of PEAR in finishing rations at 10% did not negatively impact flavor of strip steaks or consumer likability of ground beef products, but did result in changes in fatty acid composition of ground beef.

Introduction

Microalgae is currently being investigated as a potential biofuel; a barrier facing its entrance into the biofuel industry includes its inability to be cost-competitive with other petroleum-derived fuels (Pienkos and Darzins, 2009). The viability of biofuel from micro-algae is dependent upon development of a suitable market for its co-product, post-extraction algal residue (**PEAR**). Development of a market could aid in cost recovery and allow biofuel production from micro-algae to be more cost-competitive and sustainable.

Post-extraction algal residue contains a high proportion of protein (18-38% CP; Bryant et al., 2012) which could be included in finishing rations of beef steers as an energy source similar to inclusion of co-products of other industries (distillers' grains (**DG**), cottonseed meal, bakers' by-products, etc.).

Before including PEAR in beef finishing rations it is necessary to ensure that there are no negative effects from feeding the co-product that are transferred to beef product; ingredients in finishing rations can influence beef flavor, tenderness and fatty acid composition of meat. Previous research has shown that inclusion of DG at 50% dietary DM did not affect tenderness or sensory characteristics of beef and was recommended as a viable feed alternative (Roeber et al., 2005). It is likely for PEAR to compete with DG in finishing rations, therefore it is imperative to determine if provision of PEAR affects tenderness and sensory characteristics likewise.

Currently, no information is available reporting the effects of PEAR inclusion on beef flavor, tenderness, or fatty acid composition of beef. The objectives of this study

were to determine if inclusion of PEAR in beef finishing rations caused differences in beef flavor, tenderness, and fatty acid composition compared to steers receiving infusion of glucose.

Materials and methods

The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee (IACUC 2014-0003) and the Institutional Review Board (IRB2014-0314D) at Texas A&M University.

Eighteen Angus × Hereford steers (BW = 549 ± 38.8 kg) were used in a one-way, completely randomized experiment, designed to evaluate the effects of PEAR on nutrient utilization as compared to infusions of glucose ruminally or abomasally (Chapter 2). Treatments included PEAR hand mixed into the diet (1.0 kg OM/d; PEAR), and ruminal (GR), or abomasal (GA) infusion of 1.0 kg OM/d glucose. Infused steers were ruminally cannulated, allowing continuous infusion of glucose via anchored infusion lines. Steers were adapted to housing and basal diet for 5 d; subsequently, treatments were applied for 35 d, until harvest.

Steers were housed in continuously lighted barns in individual pens and were provided *ad libitum* access to fresh water. Additionally, steers were provided a finishing ration as a basal diet at 130% voluntary intake for the duration of the study (Tables 6 and 7). The finishing ration was comprised of dry rolled corn (42.3%), ground milo (18.0%), cottonseed hulls (13.5%), grass hay (10.0%), molasses (6.7%), cottonseed meal (5.4%), vitamin/mineral premix (2.3%), urea (0.9%), and limestone (0.9%). Chemical, macromineral, micromineral, and fatty acid composition of the finishing ration and

PEAR are reported in Tables 6 and 7. After administration of treatments, steers were harvested at Rosenthal Meat Science and Technology Center on the Texas A&M University campus located in College Station, Texas.

Table 6. Chemical, macromineral, and micromineral composition of finishing ration and post-extraction algal residue (PEAR¹).

Item	Finishing Ration	PEAR ¹
DM, %	92.6	93.3
----- Dry Basis -----		
OM, %	94.3	87.8
CP, %	13.7	33.8
Ether Extract, %	3.40	3.91
Acid Hydrolysis, %	3.97	6.13
ADF, %	17.40	n.d. ²
NDF, %	33.57	n.d.
Macrominerals, %		
Ca	1.31	0.08
P	0.35	0.54
K	0.93	0.64
Mg	0.23	0.09
Na	0.30	3.16
S	0.21	0.74
Microminerals, ppm		
Al	121.6	2880.0
Co	0.83	0.83
Cu	15.85	54.70
Fe	165	3540
Mn	84.3	61.1
Mb	0.89	0.88
Zn	98.9	164.0

¹PEAR = post-extraction algal residue (*Chlorella* sp.)

²n.d. = not determined

Table 7. Fatty acid composition of finishing ration and post-extraction algal residue (PEAR¹).

Item	Finishing Ration	PEAR ¹
Fatty Acid, g/100g FAME ²		
14:0	0.26	0.72
14:1	0.05	0.41
16:0	17.32	26.19
16:1	0.29	1.99
18:0	2.36	4.12
18:1c9	21.30	37.89
18:1c11	0.65	5.32
18:2	48.63	4.47
18:3	4.85	5.03
20:0	0.03	n.f. ³
20:1	0.14	0.62
20:2	0.03	0.32
20:4	0.27	0.28
20:5	0.26	0.36
22:0	0.32	0.84
24:0	0.10	n.f.
22:6	0.10	0.11

¹PEAR = post extraction algal residue (*Chlorella* sp.)

²FAME = fatty acid methyl esters

³n.f. = none found

Phase 1: Carcass fabrication, cut selection and storage

Forty-eight h post-harvest, carcasses were partially fabricated, and Institutional Meat Purchase Specifications (**IMPS**) 180, Beef Loin, Strip Loin, Boneless (USDA, 2014) subprimals were collected from one side of each carcass receiving GA, GR, and PEAR treatments. Strip loins were further cut into top loin steaks (IMPS 1180; USDA 2014) measuring 2.54 cm in thickness. Steaks were trimmed so that external fat thickness did not exceed 0.64 cm at any point. Each steak was individually labeled and vacuum packaged. After packaging, steaks were aged for 14 d in a 4 °C cooler. Aged

samples were flash frozen at -40 °C, to prevent any effects of further aging and to minimize freezing effects on meat quality. Samples remained frozen until 1 d prior to evaluation, when steaks were removed from freezer and placed in a 4 °C cooler for thawing.

Phase 1: Sensory evaluation by expert trained panel

Steaks were cooked on an electric griddle, (Model 072306, National Presto Ind., Inc., Eau Claire, WI) set at approximately 191 °C. To monitor internal temperature of steaks during cooking, a thermometer (Omega TM HH501BT, Stamford, CT) and a 0.02 cm diameter, iron-constantan Type-T thermocouple wire were used. Thermocouple wires were inserted into the geometric center of each steak. Steaks were turned over once an internal temperature of 35 °C was reached and were removed from the grill once an internal temperature of 71 °C was reached. After cooking, samples were prepared for panelists. The ends and outer edges of each steak were trimmed away and discarded so as to serve samples from the center of each steak. Samples were cut immediately prior to serving to assure a serving temperature of 49 °C.

Strip steaks were subjected to descriptive sensory analysis, using a trained expert panel. The panelists were trained for the following flavor attributes: beef flavor identification, brown/roasted, bloody/serumy, fat-like, metallic, liver-like, green-haylike, umami, overall sweet, sweet, sour, salty, bitter, sour aromatics, animal hair, barnyard, burnt, rancid, heated oil, chemical, leather (old), apricot, green, asparagus, musty-earth/humus, cumin, floral, beet, chocolate/cocoa, medicinal, petroleum-like, smoky charcoal, smoky wood, spoiled/putrid, dairy, buttery, cooked milk, sour milk/sour dairy,

refrigerator stale, soapy, warmed over, painty, fishy, and cardboardy prior to sampling any steaks for analysis. Panel selection and training was done following the guidelines of Meilgaard et al. (2007). Panelist training consisted of 4, 2 h sessions, each occurring on separate d. During the course of training, panelists sampled reference products with provided reference flavor attributes and scores. A table in the Appendix provides definitions for each of the attributes as well as their associated references, which are in accordance with the aroma and flavor lexicon (Table 12; AMSA. 2015; Adhikari et al., 2011; Civille and Lyon, 1996).

Trained sensory panelists were presented each treatment 6 different times throughout the sensory evaluation process, which was conducted over 3 different d. No more than 12 samples were presented on any given day, including a warm up steak sample, which was presented at the beginning of each of the 3 sessions. A 10 m break was provided after half of the samples had been evaluated on each day, between samples 6 and 7. All samples were presented uniformly, after being cut into 1 cm² cubes, and served in plastic soufflé cups, each with a random 3-digit identification number. Additionally, all panelists received double distilled water and fat free ricotta cheese for cleansing of the palate between samples. Sensory evaluation was conducted in sequestered sensory booths with red light filters to prevent differences in product color from affecting the panel analysis.

Phase 2: Carcass fabrication, cut selection, and storage

Forty-eight h post-harvest, carcasses were partially fabricated, and Institutional Meat Purchase Specifications (**IMPS**) 180, Beef Loin, Strip Loin, Boneless (USDA,

2014) subprimals were collected from one side of each carcass receiving GR, and PEAR treatments. Strip loins were further cut into top loin steaks (IMPS 1180; USDA 2014) measuring 2.54 cm in thickness. Steaks were trimmed so that external fat thickness did not exceed 0.64 cm at any point. Each steak was individually labeled and vacuum packaged. After packaging, steaks were aged for 14 d in a 4 °C cooler. Aged samples were flash frozen at -40 °C, to prevent any effects of further aging and to minimize freezing effects on meat quality. Samples remained frozen until 1 d prior to evaluation, upon which steaks were removed from freezer and placed in a 4 °C cooler for thawing.

Phase 2: Tenderness evaluation by shear analysis

Strip steaks from GR and PEAR treatments were thawed in a 4 °C cooler for 24 h before cooking. Electric griddles (Model 072306, National Presto Ind., Inc., Eau Claire, WI), set at 191 °C were used to cook steaks. To monitor internal temperature of steaks during cooking, a thermometer (Omega TM HH501BT, Stamford, CT) and a 0.02 cm diameter, iron-constantan Type-T thermocouple wire were used. Thermocouple wires were inserted into the geometric center of each steak. Steaks were turned over once an internal temperature of 35 °C was reached and were removed from the grill once an internal temperature of 71 °C was reached. Steaks were allowed to cool in a 4 °C cooler for 18 h. After cooling, 6, 1.3 cm cores were removed parallel to muscle fiber orientation from each steak. Each core was sheared once, perpendicular to muscle fibers, on a United Testing machine (United 5STM-500, Huntington Beach, CA) using an 11.3 kg load cell, and a Warner-Bratzler shear force attachment. The peak force (kg) needed to

shear each core was recorded, and the mean for each steak was used in statistical analysis.

Phase 3: Carcass fabrication, cut selection, storage, and product processing

Seventy-two h post-harvest, carcasses were partially fabricated, and Beef Chuck, Chuck Roll (IMPS 116A) and Beef Round, Outside Round (Flat; IMPS 171B; USDA, 2014) subprimals were collected from one side of each carcass receiving GR and PEAR treatments. Additionally, subcutaneous adipose tissue from the round and chuck region of each carcass was trimmed off and collected. Each subprimal and adipose tissue sample was individually labeled and vacuum packaged. After packaging, steaks were held for 24 h in a 4 °C cooler. The samples were then flash frozen at -40 °C, to prevent any effects of further aging and to minimize freezing effects on meat quality. Subprimals remained frozen until 6 d prior to sensory evaluation, were removed from freezer, and placed in a 0 °C cooler for thawing. Fat samples remained frozen until 2 d prior to sensory evaluation. After thawing for 5 d (subprimals) or 1 d (adipose tissue), product was further processed into ground product.

Subprimals were first cut into smaller portions and individually placed in a table top grinder (Model 4612, Hobart Corp., Troy, OH) with a 12.5 mm plate (Hobart Corp., Troy, OH). Subcutaneous adipose tissue was also ground using the table top grinder (Model 4612, Hobart Corp., Troy, OH) and 12.5 mm plate (Hobart Corp., Troy, OH). Each ground subprimal and adipose tissue from each source were kept in separate containers, until used for further processing. Between each batch, the grinder was washed to prevent any mixing or contamination of products. After grinding, lean

(subprimal) batches were thoroughly hand mixed and a 100 g grab sample was collected and homogenized using a food processor (Custom 14, Cuisinart Corp., East Winsor, NJ). The homogenized sample was analyzed for fat content using a Rapid Fat Analyzer (Smart Trac, CEM Corp., Matthews, NC). The lean sample was aimed to be $20 \pm 2.5\%$ fat. In the event that the lean sample was out of the acceptable range, ground subcutaneous adipose tissue was added to the batch, with respect to primal location and animal. After confirmation of acceptable fat percentage or necessary reformulation, ground product was placed in a tabletop grinder (Model 4612, Hobart Corp., Troy, OH) with a 3.175 mm plate (Hobart Corp., Troy, OH). Reformulated samples were reanalyzed for fat content as previously described. After processing, 3 samples, each weighing 0.45 kg, were collected from each batch (24 total batches), individually labeled, and vacuum packaged. Two of the samples from each batch were held overnight in 4 °C cooler until sensory panel analysis was conducted and the remaining sample from each batch was frozen in a -10 °C freezer until fatty acid analysis was conducted. Processing took place over 2 d, and batches were processed with respect to randomization for consumer sensory panel.

Phase 3: Sensory evaluation by consumer sensory panel

Ninety-six consumers were recruited from the Bryan/College Station, TX community using an existing Texas A&M University consumer database, by random telephone pre-screening calls, and by the use of a recruitment flyer and email. Panelists evaluated overall, overall flavor, beef flavor, and juiciness liking, after receiving verbal instructions on how to evaluate each sample, using a 9-point scale (1 = dislike

extremely, to 9 = like extremely). Consumer demographic information for age, sex, income, household size, employment level, preference for meat cooking methods, preference for degree of doneness, other flavor profile preferences, meat consumptions levels of beef, pork, chicken, fish, eggs, and non-meat proteins at home and away from home, as well as meat shopping habits was collected from each consumer during the study. Demographic frequencies for gender, age, household income, household size, and beef consumption per week are reported in Table 8.

Table 8. Consumer panel demographic frequencies reported as percentage of respondents.

Item	Percentage of Respondents
Gender	
Male	41.67
Female	58.33
Age	
18 - 20	30.21
21 - 25	29.17
26 - 35	17.71
36 - 45	5.21
46 - 55	10.42
56 - 65	4.17
66 and older	3.13
Household income	
Below \$25,000	42.71
\$25,001 – 49,999	9.38
\$50,000 – 74,999	14.58
\$75,000 – 99,999	14.58
\$100,000 or more	18.75
Household size, number of persons	
1	18.95
2	30.53
3	22.11
4	14.74
5	6.32
6 or more	7.37
Beef consumption, meals per week	
One or two	44.79
Three or four	35.42
Five or six	15.63
Seven or more	4.17

Ground beef product was cooked in electric skillets (Model CKRVSK11, Rival, Boca Raton, FL), to an internal temperature of 71 °C. Temperature was verified using a temperature probe and thermometer (Omega [™] HH501BT, Stamford, CT) that was

inserted into the center of the largest ground beef crumbles. Consumer panelists were presented with a total of 4 samples in a random order, and received each treatment 1 time throughout the sensory evaluation process. There were a series of 4 panels conducted over 2 different d, each with 24 participants. All samples were presented uniformly, and approximately 1 oz of each ground beef product was served in plastic soufflé cups as crumbles, labeled with a random 3-digit identification number, used for product identification. Additionally, all panelists received double distilled water and saltless saltine crackers for cleansing of the palate between samples. Sensory evaluation was conducted in sequestered sensory booths with red light filters to prevent differences in product color from affecting the panel analysis.

Phase 3: Fatty acid analysis by gas chromatography

Samples were removed from the -10 °C freezer 1 d prior to analysis and placed in a 4 °C cooler to allow for thawing. Total lipids of raw ground beef were extracted by a modification of the method of Folch et al. (1957). One hundred mg of homogenized, ground beef were extracted in chloroform:methanol (2:1, v/v) and fatty acid methyl esters (**FAME**) were prepared as described by Morrison and Smith (1964), modified to include an additional saponification step (Archibeque et al., 2005). The FAME were analyzed using a Varian gas chromatograph (model CP-3800 fixed with a CP-8200 auto sampler, Varian Inc., Walnut Creek, CA). Separation of FAME was accomplished on a fused silica capillary column CP-Sil88 [100 m × 0.25 mm (i.d.)] (Chrompack Inc., Middleburg, The Netherlands), with hydrogen as the carrier gas (flow rate = 35 mL/min) (split ratio 20:1). Initial oven temperature was 150 °C; oven temperature was increased

at 5 °C/min to 220 °C and held for 22 min. Total run time was 52 min. Injector and detector temperatures were at 270 °C and 300 °C, respectively. Individual fatty acids were identified using genuine external standard GLC-68D (Nu-Chek Prep, Inc., Elysian, MN).

Statistical analyses

Phase 1: Expert trained sensory panel analysis

Expert trained sensory panel data were analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The fixed effect in the model was treatment (diet), with panel day included as a random effect. Least-squares means were calculated, pairwise comparisons were evaluated if treatment effect resulted in $P < 0.05$. A pairwise comparison was considered significant if $P \leq 0.05$.

Phase 2: Tenderness evaluation by shear analysis

Strip steak tenderness was analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The only term in the model was treatment (diet). The LSMEANS option was used to calculate treatment means. A treatment difference was considered significant if $P \leq 0.05$.

Phase 3: Consumer sensory analysis

Consumer panel data was analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The terms in the model were diet (GR or PEAR) and primal (chuck or round) and their interaction, with session number and sample order included as random effects. The LSMEANS option was used to calculate treatment means and all-pair wise comparisons. A treatment difference was considered significant if $P \leq 0.05$. A

pairwise comparison difference was considered significant if $P \leq 0.05$. An interaction was considered significant if $P \leq 0.05$. Further, demographic data collected from consumers was analyzed using the FREQ procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC).

Phase 3: Fatty acid analysis of ground beef

Fatty acid composition of ground beef was analyzed using the MIXED procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC). The terms in the model were diet (GR or PEAR) and subprimal (chuck or round) and no random effects were included in the model. The LSMEANS option was used to calculate treatment means and all pair wise comparisons as stated above.

Results

Phase 1: Flavor of strip steaks

Beef flavor attributes of strip steaks from steers receiving GA, GR, and PEAR treatments are reported in Table 9. There was an observed treatment difference in fat-like flavor ($P = 0.02$); however, PEAR strip steaks were not different from either GA or GR ($P > 0.10$) steaks, but GA and GR steaks were different from one another in fat-like flavor ($P < 0.01$). Additionally, overall sweet flavor and aroma was different among treatments ($P = 0.05$); PEAR feeding resulted in greater overall sweet flavor and aroma than feeding GA (0.58 vs. 0.30; $P < 0.02$). Sweet flavor was also different among treatments ($P < 0.01$), but was not different between GA and GR ($P < 0.20$); however, PEAR was sweeter than GA ($P < 0.01$) and GR ($P < 0.02$).

Table 9. Beef flavor attributes of strip steaks from steers that consumed post-extraction algal residue (PEAR¹) or were infused with glucose.²

Attribute	Treatments ³			SEM	P-value
	GA	GR	PEAR		
Beef ID	5.10	5.13	5.10	0.12	0.98
Brown/Roasted	1.10	1.23	1.02	0.23	0.78
Bloody/Serumy	1.23	1.23	1.33	0.17	0.90
Fat-like	0.87 ^a	1.30 ^b	1.07 ^{a,b}	0.13	0.02
Metallic	1.83	1.70	1.76	0.11	0.58
Umami	0.57	0.37	0.69	0.15	0.19
Overall Sweet	0.30 ^a	0.50 ^{a,b}	0.58 ^b	0.08	0.05
Sweet	0.13 ^a	0.23 ^a	0.44 ^b	0.08	< 0.01
Sour	2.00	1.87	2.13	0.15	0.46
Salty	1.23	1.47	1.50	0.10	0.17
Bitter	1.87	1.80	1.83	0.12	0.92
Burnt	0.07	0.10	0.00	0.06	0.13

¹PEAR = post extraction algal residue (*Chlorella* sp.)

²Within each row, means with differing subscripts differ ($P \leq 0.05$)

³GA = 1.0 kg OM glucose infused into the abomasum daily; GR = 1.0 kg OM glucose infused into the rumen daily; PEAR = 1 kg OM post-extraction algal

Provision of PEAR in finishing rations of beef steers did not result in significant or numeric differences in beef identification, brown/roasted, bloody/serumy, metallic, umami, sour, salty, bitter, or burnt flavor attributes compared to steers receiving infused glucose ($P \geq 0.13$).

None of the following flavor attributes were detected in any of the steaks from GA, GR, or PEAR treatments: liver-like, green-haylike, sour aromatics, animal hair, barnyard, rancid, heated oil, chemical, leather (old), apricot, green, asparagus, musty-earth/humus, cumin, floral, beet, chocolate/cocoa, medicinal, petroleum-like, smoky

charcoal, smoky wood, spoiled/putrid, dairy, buttery, cooked milk, sour milk/sour dairy, refrigerator stale, soapy, warmed over, painty, fishy, and cardboardy. Means for the flavor attributes not found to be present were 0, and are not shown.

Phase 2: Tenderness evaluation by shear analysis

No significant or numeric differences in WBSF values were observed between GR (2.77 kg) or PEAR (2.50 kg; $P = 0.25$).

Phase 3: Flavor and fatty acid composition of ground beef

No diet \times primal interactions were observed ($P \geq 0.15$; Table 10). Furthermore, there was no effect of diet (PEAR or GR) on overall, overall flavor, beefy flavor, or juiciness liking of ground beef by consumers ($P \geq 0.58$). Additionally, there was no effect of primal (chuck or round) on overall like, overall flavor like, beefy flavor like, or juiciness like ($P \geq 0.17$). However, ground beef from the round was better liked (6.54) than ground beef from the chuck (6.15; $P = 0.04$); but while this difference is statistically different, the magnitude of the difference indicates little meaningful segregation of these products in the marketplace.

Table 10. Consumer sensory ratings for ground beef from steers fed post-extraction algal residue (PEAR¹) or infused with glucose².

Item	Diet ³		Primal		SEM	<i>P</i> -values	
	GR	PEAR	Chuck	Round		Diet	Primal
Overall Liking	6.40	6.30	6.25	6.45	0.23	0.58	0.24
Overall Flavor	6.28	6.19	6.11	6.37	0.23	0.65	0.17
Liking							
Beefy Flavor	6.38	6.31	6.15	6.54	0.28	0.71	0.04
Liking							
Juiciness Liking	6.20	6.20	6.21	6.20	0.27	0.98	0.97

¹PEAR = post-extraction algal residue

²No diet × primal interactions were observed ($P > 0.05$).

³GR = 1.0 kg OM glucose infused into the rumen daily; PEAR = 1 kg OM post-extraction algal residue fed daily

The only diet × primal interaction ($P = 0.02$) was for palmitoleic acid concentration in ground beef; PEAR ground round (4.50%) had more palmitoleic acid than PEAR ground chuck (2.92%) and GR ground round (3.78%) had more palmitoleic acid than GR ground chuck (2.93%; Table 11). Compared to GR ground beef, supplementation of PEAR increased myristic, palmitic, and eicosapentaenoic acid (EPA) concentration ($P \leq 0.03$), while oleic acid concentration decreased ($P < 0.01$). Myristic, myristoleic, palmitoleic, oleic, and eicosapentaenoic acid were at higher concentrations ($P \leq 0.02$), while stearic acid was lower (11.99 vs. 16.05%; $P < 0.01$) in ground beef from the round than from the chuck. Inclusion of PEAR in finishing rations of beef steers resulted in increased concentration of myristic, palmitic, palmitoleic acids, and EPA in ground beef product compared to GR ground beef ($P \leq 0.03$), while there was a decrease in oleic acid concentration ($P < 0.01$). Of the differences observed for diet, palmitic and oleic acid were the most affected; there were approximately 2 g/100 g

FAME more of palmitic acid and 2 g/100 g FAME less of oleic acid in PEAR-fed ground beef compared to ground beef from those receiving GR. Myristic, myristoleic, palmitoleic, oleic, and EPA was found at higher concentrations in ground beef from the round compared to ground chuck ($P \leq 0.02$). However, there was a decrease in stearic acid in ground beef from the round compared to the chuck (11.99 vs. 16.05; $P < 0.01$). Similar to the difference observed in GR and PEAR ground beef, there was a 2 g/100 g FAME difference of oleic acid in ground beef from the round compared to the chuck.

Table 11. Fatty acid composition (% FAME¹) of ground beef from steers fed post-extraction algal residue (PEAR²) or infused with glucose.

Item	Diet ³		Primal		SEM	<i>P</i> -values	
	GR	PEAR	Chuck	Round		Diet	Primal
14:0	3.33	3.93	3.36	3.90	0.16	0.02	0.02
14:1	0.68	0.92	0.57	1.02	0.10	0.09	< 0.01
16:0	27.62	29.56	28.30	28.88	0.35	< 0.01	0.26
16:1 ⁴	3.36	3.71	2.92	4.14	0.11	0.02	< 0.01
18:0	14.52	13.50	16.05	11.99	0.37	0.07	< 0.01
18:1c9	38.68	36.32	36.49	38.52	0.53	< 0.01	< 0.01
18:2	2.58	2.74	2.82	2.50	0.24	0.65	0.36
18:3	0.28	0.32	0.33	0.27	0.02	0.20	0.12
20:1	0.09	0.10	0.10	0.09	0.02	0.69	0.56
20:2	0.07	0.10	0.07	0.10	0.01	0.03	0.02
20:4	0.11	0.12	0.12	0.10	0.01	0.44	0.38

¹FAME = Fatty acid methyl esters

²PEAR = post-extraction algal residue

³GR = 1.0 kg OM glucose infused into the rumen daily; PEAR = 1 kg OM post-extraction algal residue fed daily

⁴A diet × primal interaction occurred ($P = 0.02$). No other diet × primal interactions were observed ($P > 0.05$).

Discussion

Phase 1: Flavor of strip steaks

No off-flavors in strip steaks were detected from inclusion of PEAR in finishing rations of beef steers, and only minor differences were observed for differences in fat-like, overall sweet, and sweet flavors between the three treatments, so it can be hypothesized that in lean cuts of beef, there will not be flavor differences in PEAR-fed beef of magnitude for the consumer to detect.

Phase 2: Tenderness evaluation by shear analysis

Warner-Bratzler Shear Force values from inclusion of PEAR and GR were below the levels to be considered “very tender,” as established by Destefanis et al. (2008), because mean shear force values were under 3.2 kg for both treatments (Table 10). Additionally, results are similar to results of Kroger et al. (2004) and Roeber et al. (2005) who found that inclusion of distillers’ grains in finishing diets at 20-50% dietary inclusion did not result in differences in WBSF. However, further investigation of PEAR at higher levels of inclusion in diets of finishing steers should be observed because dietary inclusion was only approximately 9% dietary DM to ensure increased levels of inclusion did not impact tenderness.

Fat content plays a role in tenderness and flavor of beef. In a study by Smith et al. (1985), it was concluded that carcasses with higher QG produced more tender and palatable cuts, and there was less variability among cuts with higher QG. Small, but significant increases were found in juiciness, tenderness, and flavor as marbling score improved from practically devoid to moderately abundant. When comparing practically

devoid and moderately abundant, steaks from the loin were more palatable 66% of the time and differences in marbling explained 33% of the variation in overall palatability (Smith et al., 1985). In our study, it is likely that no differences in tenderness and only minor differences in beef flavor were found in strip steaks because there were only minor (68 degrees of marbling), but significant ($P = 0.01$) differences in QG and marbling scores (data reported in Chapter 2; Table 5).

Phase 3: Flavor and fatty acid composition of ground beef

No differences were observed in either GR or PEAR ground beef for overall, overall flavor, beefy flavor, or juiciness liking when assessed by consumers. Therefore, provision of PEAR at 1.0 kg OM/d is not likely to be detrimental to palatability of cooked beef. Similarly, when distillers' grains were fed to finishing Holstein steers at increasing levels, it was determined that distillers' grains could be included in finishing rations ideally at 10-25% dietary DM, without having effects on cooked beef palatability (Roeber et al., 2005). In a consumer sensory study, when wet distillers' grains were included at 50% dietary DM, 51% of consumers were not pleased with the samples they tasted, compared to only 30% who were not pleased at 10% dietary DM inclusion (Roeber et al., 2005). As previously mentioned, it would be worthwhile to investigate PEAR at higher levels of inclusion in diets of finishing steers because in this study, PEAR was only included at approximately 9% dietary DM.

However, it is not likely that PEAR will be included in finishing rations at extremely high percentages because of the palatability associated with PEAR due to its Na content (3.16%; Table 6), which is a self-limiting property. Additionally, PEAR

contains a high level of CP (33.8% DM); CP in finishing rations for beef cattle can typically range from 12.5 to 14.4%, according to a survey of consulting nutritionists (Galyean, 1996). Furthermore, PEAR contains high levels of Al (2880 ppm) and Fe (3540 ppm), so PEAR will be required to be formulated into rations at appropriate levels with other ingredients containing Al and Fe, so as to not exceed NRC (2000) requirements; (DM basis; Table 1).

Previous studies have attempted to measure the differences in fatty acid composition of beef from cattle fed forages compared to concentrates, but animals with *ad libitum* access to concentrate rations tended to have heavier and fatter carcasses compared to those finished on forages for determined periods of time (Hidioglou et al., 1987; Mandell et al., 1998). The cause of heavier, fatter carcasses is likely due to the fact that those animals consuming concentrate rations had higher energy intake, as concentrates are more energy dense, compared to their counterparts consuming forages. A greater amount of MUFA are typically found in the subcutaneous fat component of fatter animals (Leat, 1978). Changes in carcass fatness can confound effects of ration type on fatty acid composition (French et al., 2000). In our study, during the finishing phase prior to harvest, dietary energy intake was not different among treatments ($P = 0.45$) and averaged 36 Mcal/d, but did however make the diet less energy dense per kg of intake compared to glucose (data reported in Chapter 2, Table 3). Additionally, HCW and fat thickness of carcasses was not different among treatments (data reported in Chapter 2, Table 5).

Of the fatty acids present in the PEAR supplement, palmitic, oleic, and α -linolenic acid were the most abundant SFA, MUFA, and PUFA at 26.2, 37.9, and 5.0 g/100 g FAME, respectively. Of palmitic, oleic, and α -linolenic acid; palmitic acid was the only fatty acid that significantly increased as a result of diet in ground beef from the round and chuck. It was hypothesized that if PEAR bypassed the rumen, the fatty acids present in PEAR would not undergo biohydrogenation and would be seen at increased levels in beef from PEAR fed steers. Increased oleic acid consumption with PEAR supplementation resulted in decreased oleic acid content of ground beef, therefore, it is likely that PEAR does not bypass the rumen during digestion; however, this should be confirmed in later *in-vivo* or *in-vitro* studies.

It is questionable whether the higher concentration of the MUFA, myristoleic and palmitoleic acid, found in ground beef from the round resulted from dietary differences in myristoleic and palmitic acid (Chapter 2, Table 4) or because greater amounts of MUFA are typically found in subcutaneous fat (Leat, 1978) which could have been added to the ground product during formulation. During preparation of ground beef, batches were formulated to contain $20 \pm 2.5\%$ fat and as such subcutaneous fat was added to each batch accordingly. The chuck roll contains several muscles, separated by intermuscular fat, which would have been present in the ground portion of the chuck subprimal (USDA, 2014). Whereas the round subprimal is likely to have contained less intermuscular fat (USDA, 2014). Therefore, a greater amount of subcutaneous fat from the round was likely to have been added to the ground round product from both GR and

PEAR treatments since all ground beef batches in this study were formulated to contain $20 \pm 2.5\%$ fat.

Conclusion

The purpose of this experiment was to determine if feeding PEAR: 1) produced off-flavors in steaks as evaluated by an expert, trained sensory panel, 2) caused differences in tenderness of strip steaks, 3) produced differences in flavor in ground round and ground chuck detectable by the average consumer, and 4) resulted in differences in fatty acid composition of ground beef that could potentially impact flavor. Supplementation of PEAR fed at the level of 1kg OM/d did not result in off flavors in strip steaks according to a trained panel or flavor differences in ground beef able to be detected by consumers. As there were no detrimental effects to beef palatability due to provision of PEAR, it should be further investigated as a source of protein for livestock, even finishing steers. Additionally, inclusion of PEAR at higher dietary levels and its effect on beef flavor, tenderness, and fatty acid composition should be studied.

CHAPTER IV

CONCLUSIONS

The purpose of these experiments were to: 1) determine the impact of PEAR on nutrient utilization, 2) compare the impacts of treatments on carcass characteristics, 3) determine if PEAR produced off-flavors in steaks as evaluated by an expert, trained sensory panel, 4) identify differences in tenderness of strip steaks, 5) determine if consumers could detect difference in flavor of ground round and ground chuck, and 6) discover if potential differences in flavor were a result of different fatty acid composition of beef. The inclusion of PEAR caused digestibility of the diet to decrease.

The results of these studies suggest that PEAR could be included at the rate of 1.0 kg/d OM to provide increased USDA Quality Grade, an important carcass value determining characteristic without decreasing intake and alternating USDA Yield Grade. The optimum inclusion level of PEAR for impact on nutrient utilization and carcass value determining characteristics, however should be further investigated. Inclusion of PEAR did not result in flavor differences in strip steaks according to a trained panel or flavor differences in ground beef able to be detected by consumers. As there were no detrimental effects to beef palatability due to provision of PEAR, it should be further investigated as a source of protein for livestock, even finishing steers. Additionally, inclusion of PEAR at higher dietary levels and its effect on beef flavor, tenderness, and fatty acid composition should be studied.

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APPENDIX

Table 12. Sensory attributes, their definitions and their associated references, which are in accordance with the aroma and flavor lexicon used for the trained sensory panel.

Sensory attribute	Definition	Reference(s) and standard flavor scale value(s)
Barnyard	Combination of pungent, slightly sour, hay-like aromatics associated with farm animals and the inside of a horn.	White pepper in water = 4.5 (aroma) Tincture of civet = 6.0 (aroma)
Beef identity	Amount of beef flavor identity in the sample.	Swanson's beef broth = 5.0 (aroma and flavor) 80% lean ground beef = 7.0 (aroma and flavor) Beef brisket – 11.0 (aroma and flavor)
Bitter	The fundamental taste factor associated with a caffeine solution.	0.01% caffeine solution = 2.0 (flavor) 0.02% caffeine solution = 3.5 (flavor)
Bloody/serumy	The aromatics associated with blood on cooked meat products. Closely related to metallic aromatic.	USDA Choice strip stea = 5.5 (aroma and flavor)
Brown/roasted	A round, full aromatic generally associated with beef suet that has been broiled.	Beef suet = 8.0 (aroma and flavor) 80% lean ground beef = 10.0 (aroma and flavor)
Burnt	The sharp/acrid flavor note associated with over-roasted beef muscle, something over-baked or excessively browned in oil.	Alf's red wheat puffs = 5.0 (aroma and flavor)
Cardboardy	The fundamental taste factor associated with cardboard.	Wet cardboard = 6.0 (aroma and flavor)

Table 12 continued.

Chemical	The aromatics associated with garden hose, hot Teflon pan, plastic packaging and petroleum based product such as charcoal lighter fluid.	Zip-Loc sandwich bag = 13.0 (aroma) Clorox in water = 6.5 (flavor)
Cocoa	The aromatics associated with cocoa beans and powdered cocoa and chocolate bars. Brown, sweet, dusty, often bitter aromatics.	Hershey's cocoa powder in water = 3.0 (flavor) Hershey's chocolate Kiss = 7.5 (aroma), 8.5 (flavor)
Dairy	The aromatics associated with products made from cow's milk, such as cream, milk, sour cream, or butter milk.	Dillon's reduced fat milk (2%) = 8.0 (flavor)
Fat-like	The aromatics associated with cooked animal fat.	Hillshire farms Litl' beef smokies = 7.0 (aroma) Beef suet = 12.0 (aroma and flavor)
Fishy	The aromatics associated with some rancid fats and oils (similar to old fish).	Catfist = 15 (flavor)
Green/hay-like	Sharp, slightly pungent aromatics associated with green/plant/vegetable matters such as parsley, spinach, pea pods, fresh cut grass, etc.	Hexanal in propylene glycol (5,000 ppm) = 6.5 (aroma) Fresh parsley in water = 9.0 (flavor)
Heated oil	The aromatics associated with oil heated to a high temperature.	Lay's potato chips = 4.0 (aroma) Wesson vegetable oil = 7.0 (flavor)
Liver-like	The aromatics associated with cooked organ meat/liver.	Beef liver = 7.5 (aroma and flavor) Braunschweiger liver sausage = 10.0 (aroma and flavor; must taste and swallow)
Medicinal	A clean sterile aromatic characteristic of antiseptic like products such as Band-Aids, alcohol, and iodine.	Band-Aid = 6.0 (aroma)

Table 12 continued.

Metallic	The impression of slightly oxidized metal, such as iron, copper, and silver spoons.	0.10% potassium chloride solution = 1.5 (flavor) USDA Choice strip steak = 4.0 (aroma and flavor) Dole canned pineapple juice = 6.0 (aroma and flavor)
Musty/moldy/humus	Musty, sweet, decaying vegetation.	Asparagus in water = 6.5 (flavor)
Overall sweet	A combination of sweet taste and sweet aromatics. The aromatics associated with the impression of sweet.	Post shredded wheat (spoon size) = 1.5 (flavor) Hillshire Farms Litl' beef smokies = 3.0 (flavor) SAFC ethyl maltol (99%) = 4.5 (aroma) Linseed oil = 15 (aroma)
Painty	The aromatic commonly associated with rancid oil and fat (distinctly like linseed oil).	Linseed oil = 15 (aroma)
Rancid	The aromatics commonly associated with oxidized fat and oils. These aromatics may include cardboard, painty, varnish, and fishy.	Wesson vegetable oil (microwaved 3 m) = 7.0 (flavor) Wesson vegetable oil (microwaved 5 m) = 9.0 (flavor)
Salty	The fundamental taste factor of which sodium chloride is typical.	0.15% NaCl solution = 1.5 (flavor) 0.25% NaCl solution = 3.5 (flavor)
Smokey wood	Dry, dusty, aromatic reminiscent of burning wood.	Wright's Natural Hickory seasoning in water = 7.5 (aroma)
Sour	The fundamental taste factor associated with citric acid.	0.015% citric acid solution = 1.5 (flavor)
Sour aromatics	The aromatics associated with sour substances.	Dillon's buttermilk = 5.0 (flavor)

Table 12 continued.

Sour milk/dairy	Sour, fermented aromatics associated with dairy products such as buttermilk and sour cream.	Laughing cow light Swiss cheese = 3.0 (aroma), 7.0 (flavor) Dillon's buttermilk = 4.0 (aroma); 9.0 (flavor)
Spoiled	The presence of inappropriate aromatics and flavors that is commonly associated with products. It is a foul taste and/or smell that indicates the product is starting to decay and putrefy. The fundamental taste factor associated with sucrose.	Dimethyl disulfide in propylene glycol (10,000 ppm) = 12.0 (aroma)
Sweet	The fundamental taste factor associated with sucrose.	2.0% sucrose solution = 2.0 (flavor)
Umami	Flat, salty, somewhat brothy. The taste of glutamate, salts of amino acids. and other molecules called nucleotides.	0.035% accent flavor enhancer solution = 7.5 (flavor)